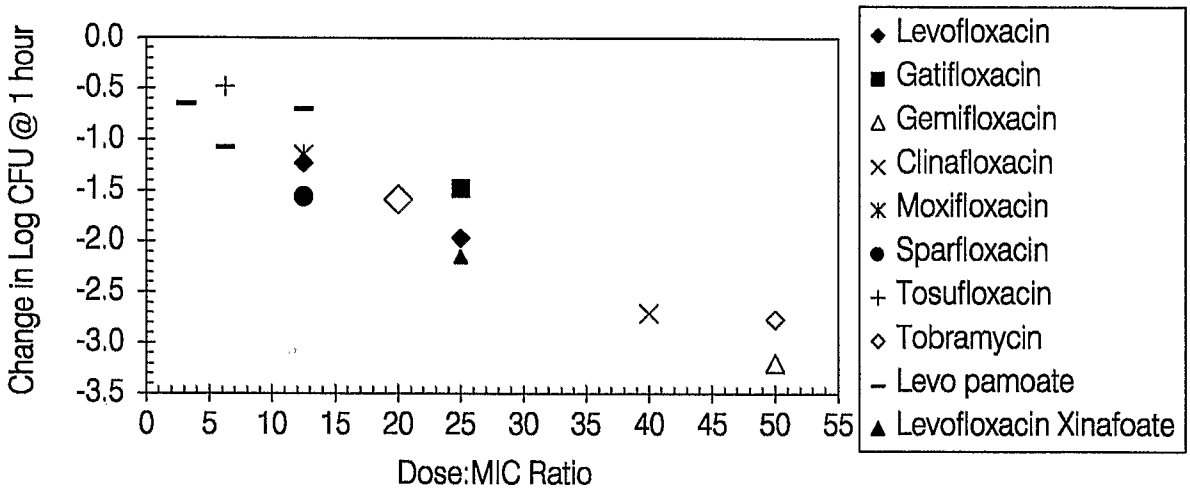

Change in Log CFU @ 1 hour
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
0 5 10 15 20 25 30 35 40 45 50 55
Dose:MIC Ratio
Levofloxacin
Gatifloxacin
Gemifloxacin
Clinafloxacin
Moxifloxacin
Sparfloxacin
Tosufloxacin
Tobramycin
Levo pamoate
Levofloxacin Xinafoate

FIG. 1

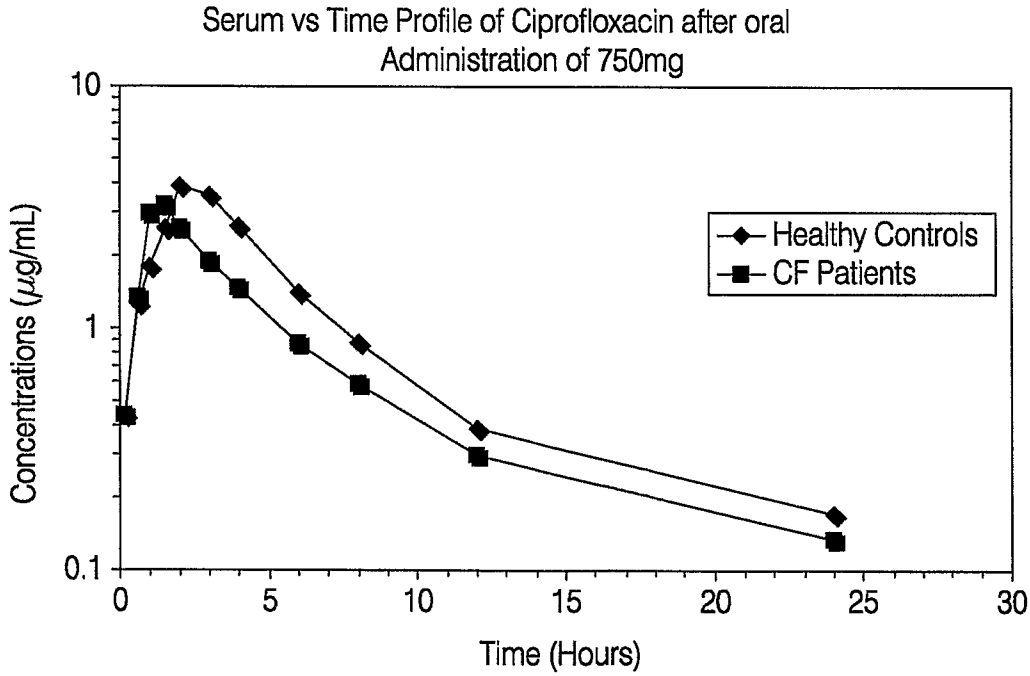

Serum vs Time Profile of Ciprofloxacin after oral Administration of 750mg
Concentrations (μg/mL)
10
1
0.1
0 5 10 15 20 25 30
Time (Hours)
Healthy Controls
CF Patients

FIG. 2

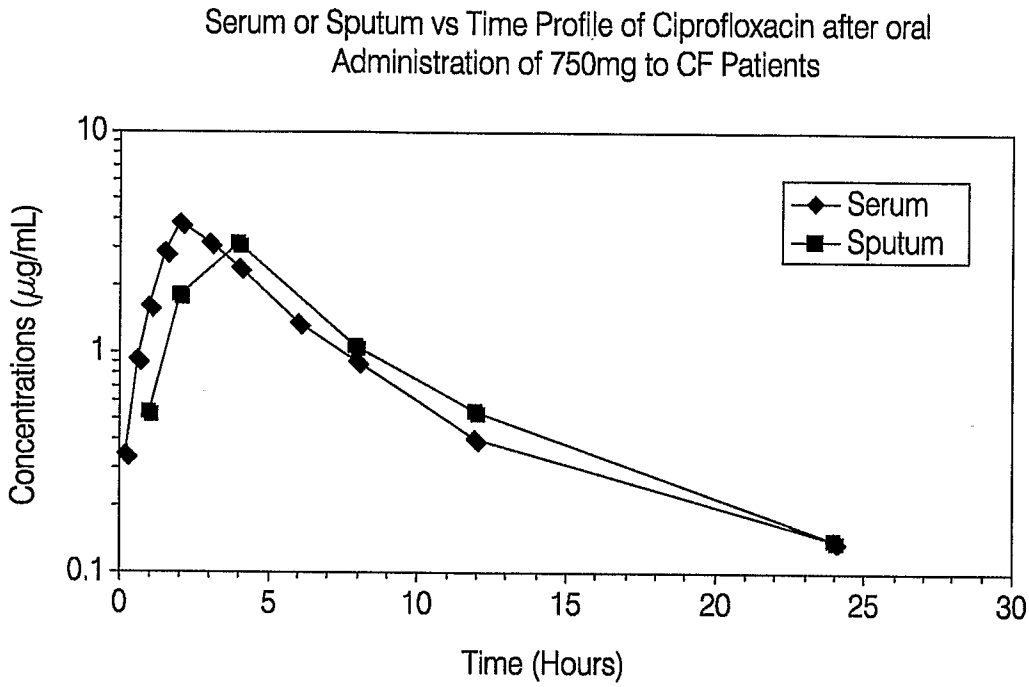

Serum or Sputum vs Time Profile of Ciprofloxacin after oral Administration of 750mg to CF Patients
Concentrations (μg/mL)
10
1
0.1
0
5
10
15
20
25
30
Time (Hours)
Serum
Sputum

FIG. 3

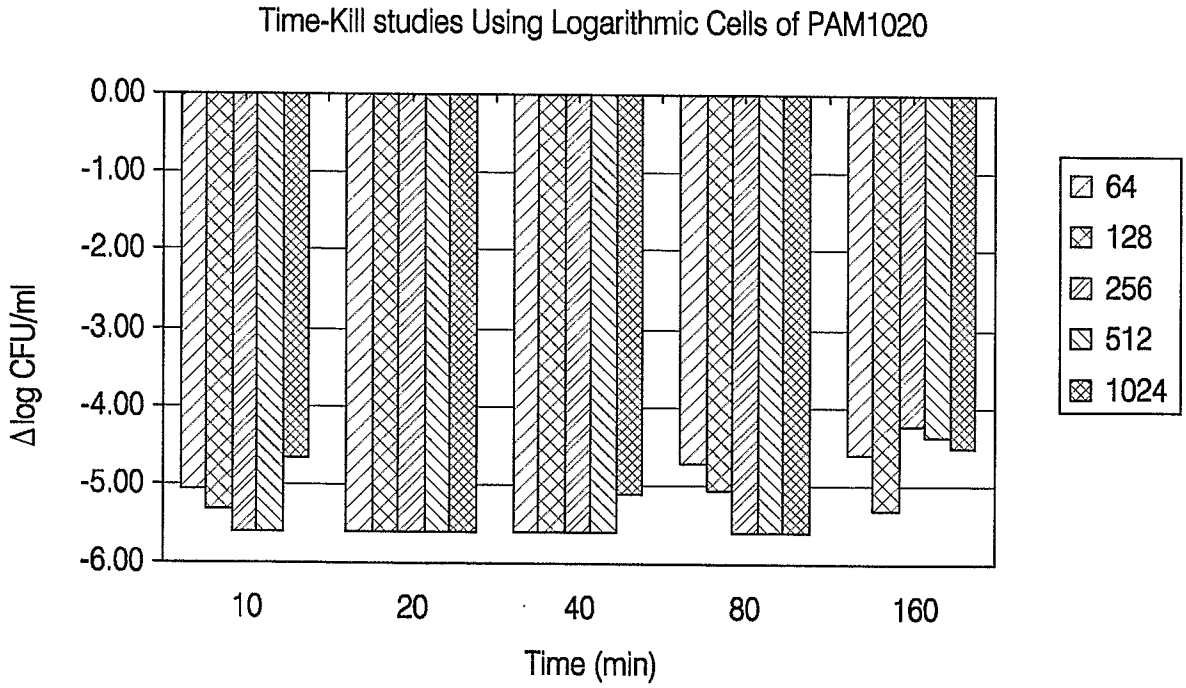

Time-Kill studies Using Logarithmic Cells of PAM1020
Δlog CFU/ml
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
-6.00
10
20
40
80
160
Time (min)
64
128
256
512
1024

FIG. 4A

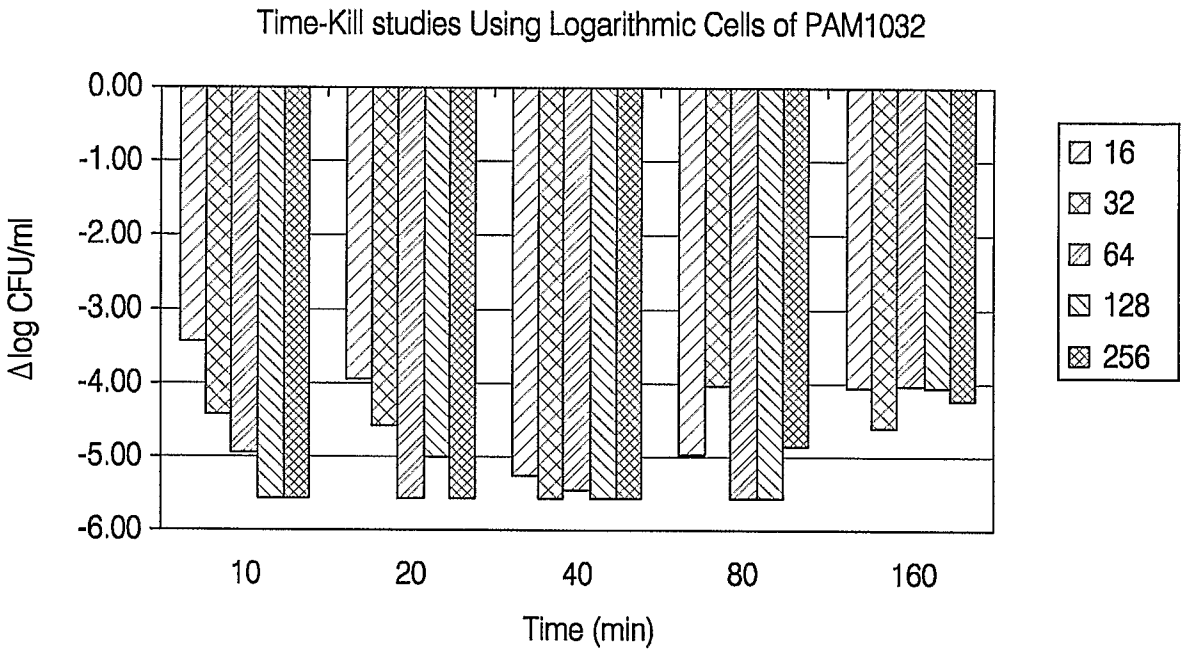

Time-Kill studies Using Logarithmic Cells of PAM1032
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
-6.00
Δlog CFU/ml
10
20
40
80
160
Time (min)
16
32
64
128
256

FIG. 4B

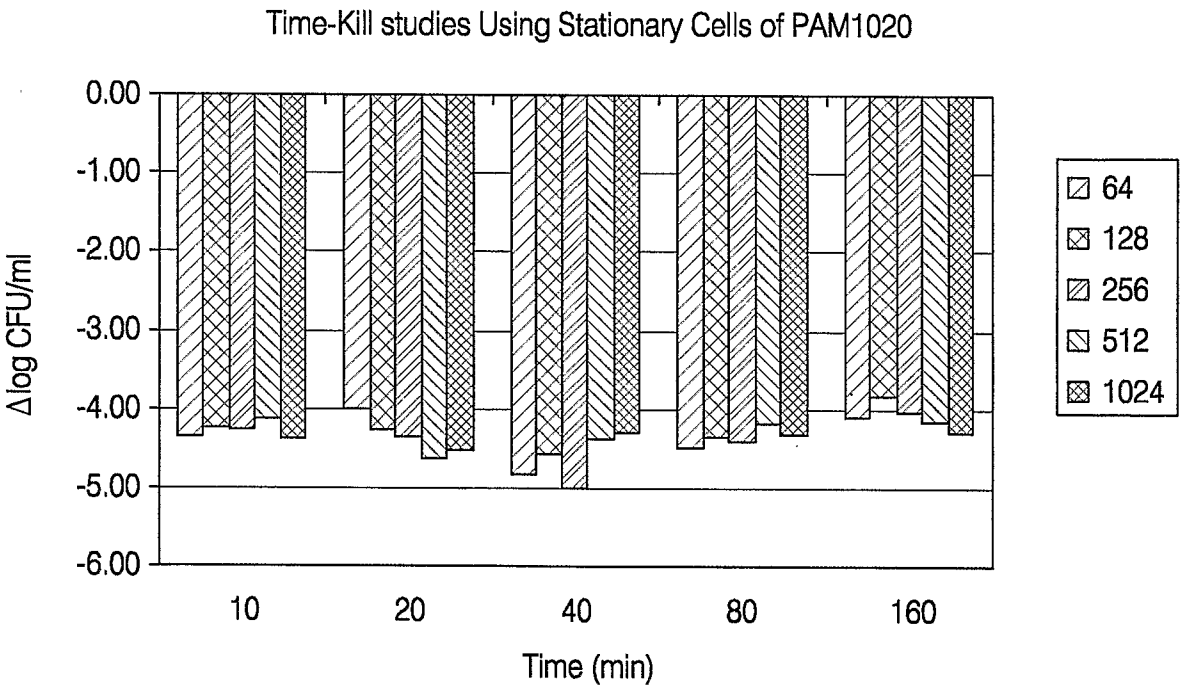

Time-Kill studies Using Stationary Cells of PAM1020
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
-6.00
Δlog CFU/ml
10
20
40
80
160
Time (min)
64
128
256
512
1024

FIG. 5A

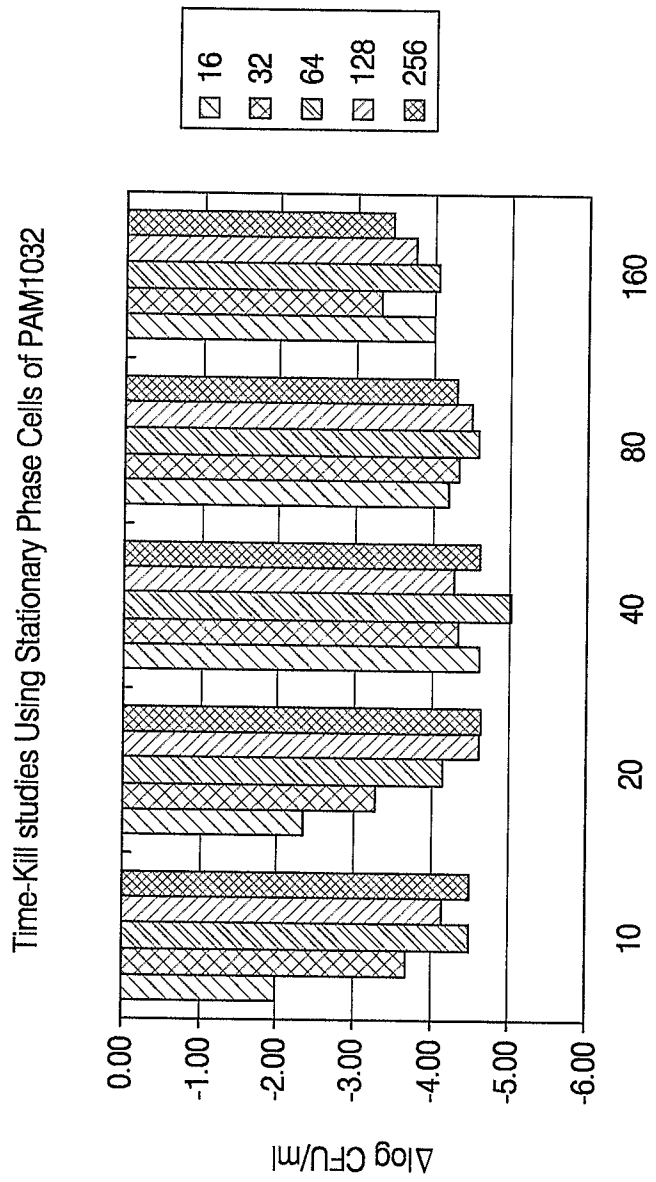

Time-Kill studies Using Stationary Phase Cells of PAM1032
16
32
64
128
256
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
-6.00
Δlog CFU/ml
10
20
40
80
160
Time (min)

FIG. 5B

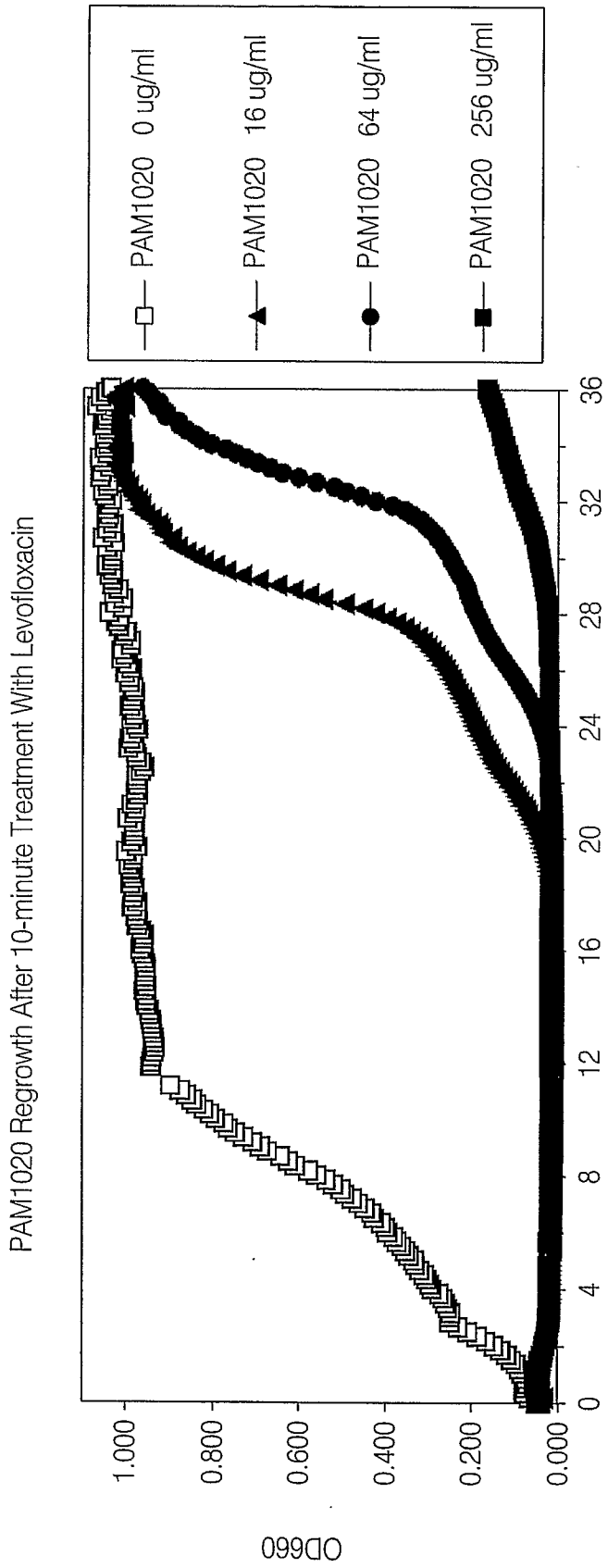

PAM1020 Regrowth After 10-minute Treatment With Levofloxacin
PAM1020 0 ug/ml
PAM1020 16 ug/ml
PAM1020 64 ug/ml
PAM1020 256 ug/ml
OD660
1.000
0.800
0.600
0.400
0.200
0.000
0
4
8
12
16
20
24
28
32
36
Time (hour)

FIG. 6A

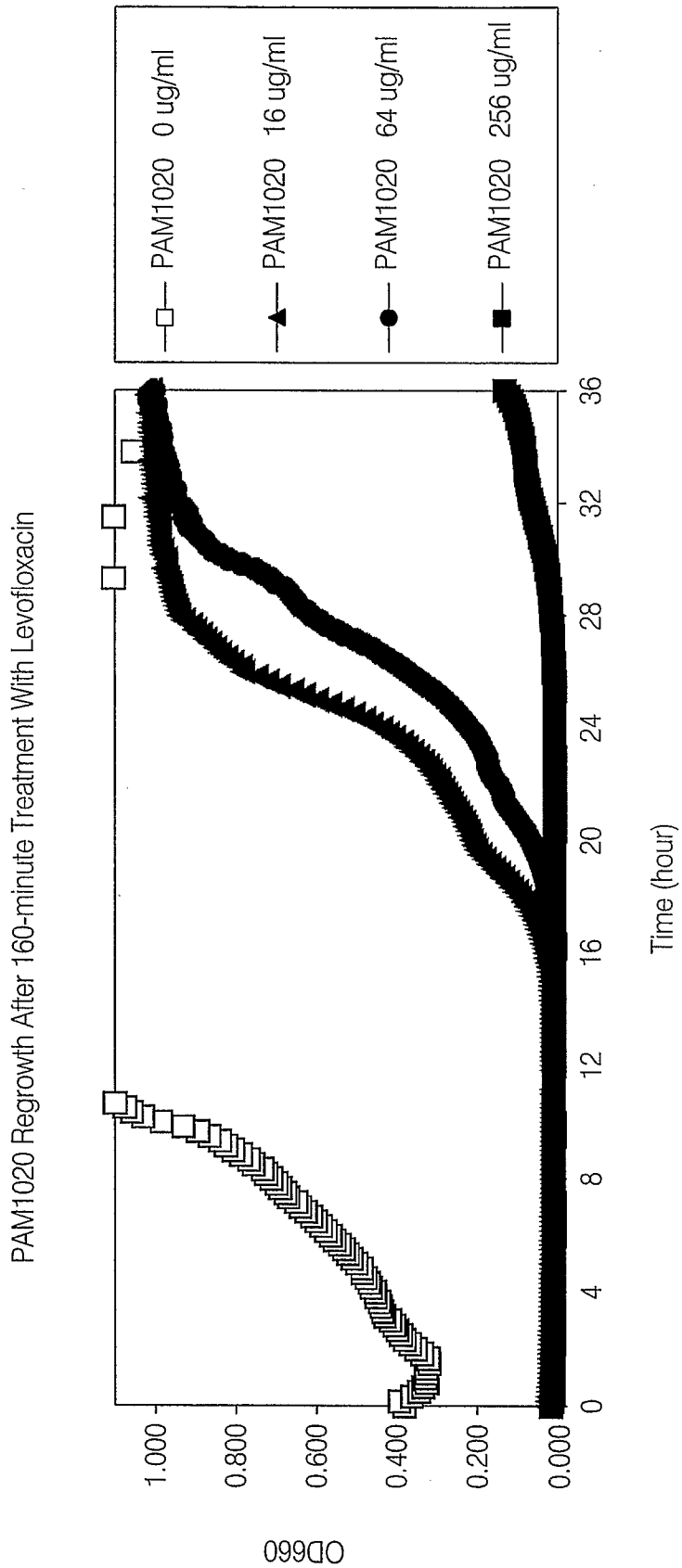

PAM1020 Regrowth After 160-minute Treatment With Levofloxacin
PAM1020 0 ug/ml
PAM1020 16 ug/ml
PAM1020 64 ug/ml
PAM1020 256 ug/ml
OD660
1.000
0.800
0.600
0.400
0.200
0.000
0
4
8
12
16
20
24
28
32
36
Time (hour)

FIG. 6B

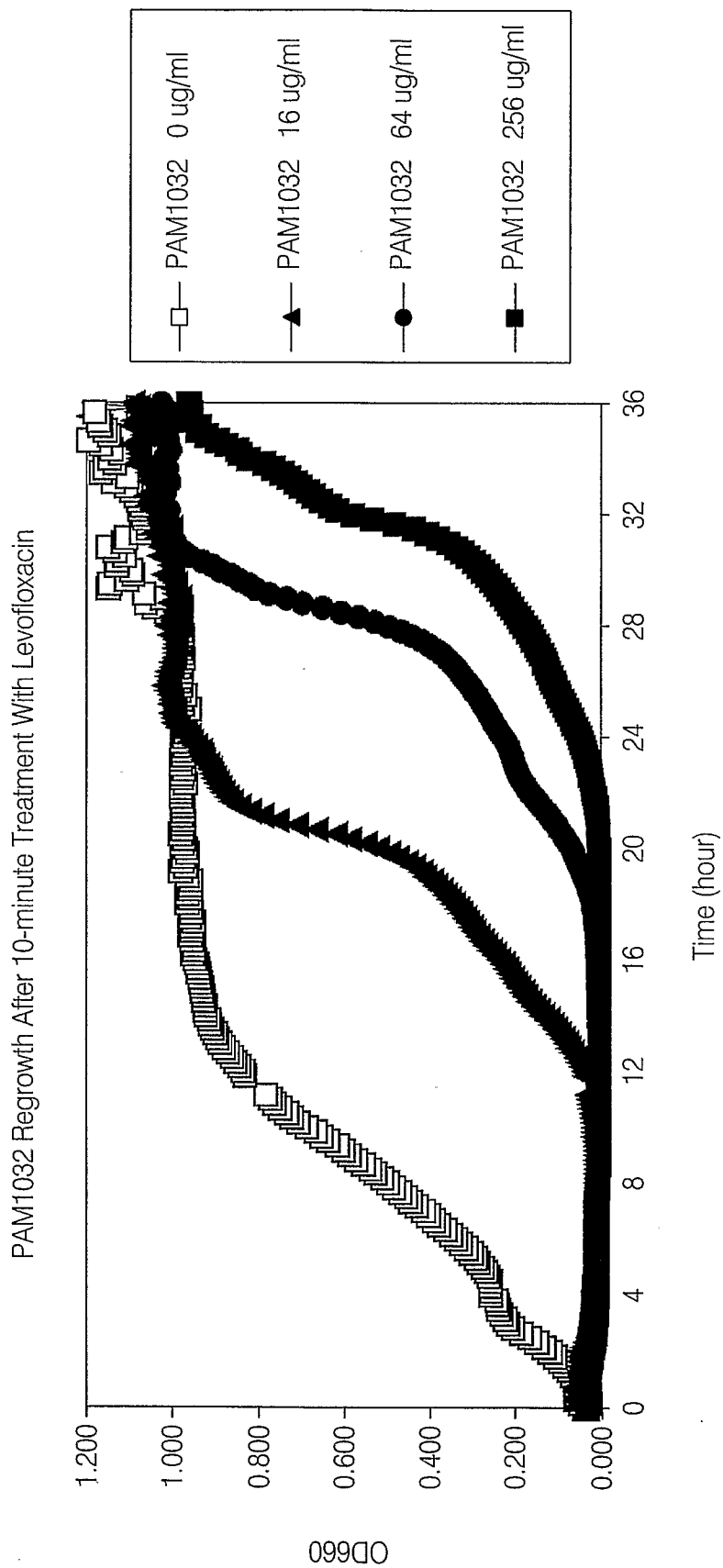

PAM1032 Regrowth After 10-minute Treatment With Levofloxacin
PAM1032 0 ug/ml
PAM1032 16 ug/ml
PAM1032 64 ug/ml
PAM1032 256 ug/ml
OD660
1.200
1.000
0.800
0.600
0.400
0.200
0.000
0
4
8
12
16
20
24
28
32
36
Time (hour)

FIG. 6C

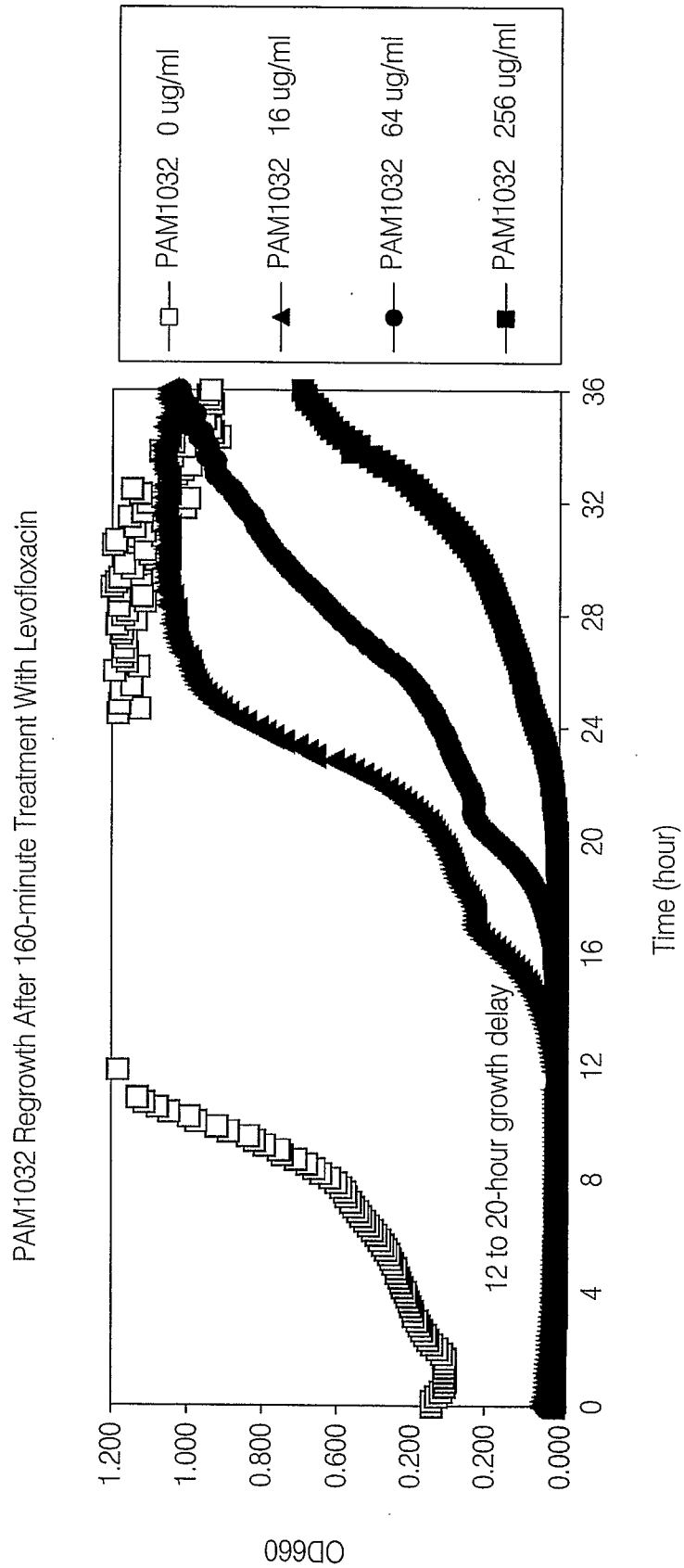

PAM1032 Regrowth After 160-minute Treatment With Levofloxacin
PAM1032 0 ug/ml
PAM1032 16 ug/ml
PAM1032 64 ug/ml
PAM1032 256 ug/ml
12 to 20-hour growth delay
OD660
Time (hour)
1.200
1.000
0.800
0.600
0.200
0.200
0.000
0
4
8
12
16
20
24
28
32
36

FIG. 6D

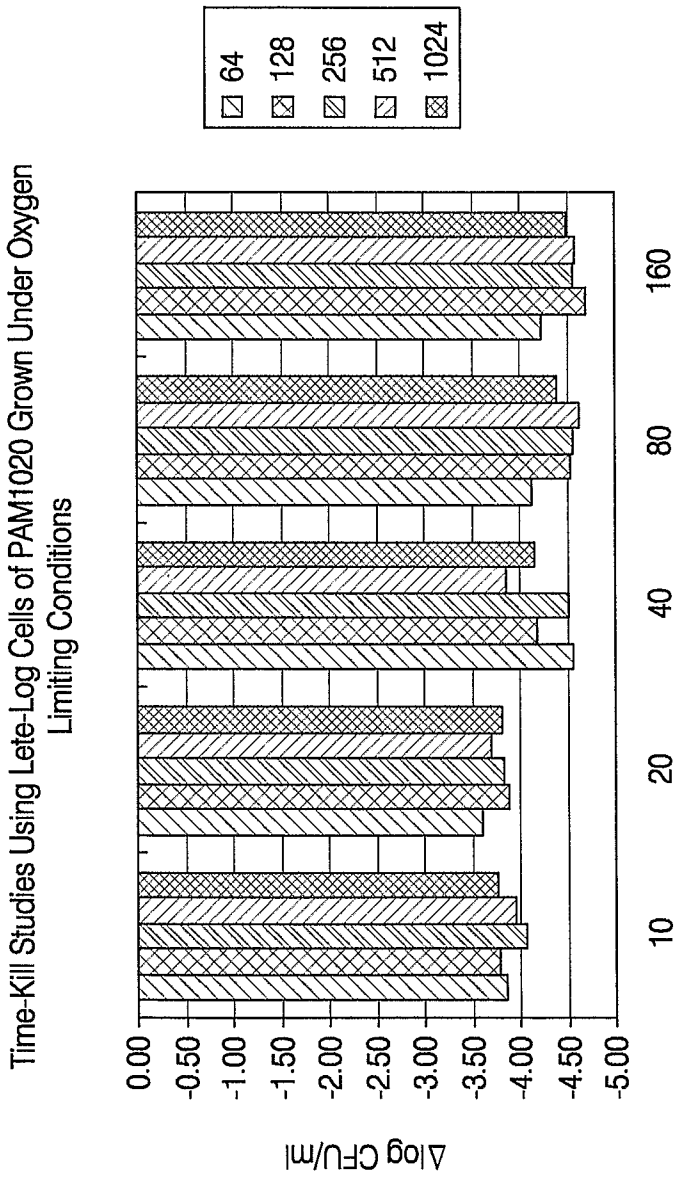

Time-Kill Studies Using Lete-Log Cells of PAM1020 Grown Under Oxygen Limiting Conditions
64
128
256
512
1024
0.00
-0.50
-1.00
-1.50
-2.00
-2.50
-3.00
-3.50
-4.00
-4.50
-5.00
Δlog CFU/ml
10
20
40
80
160
Time (min)

FIG. 7A

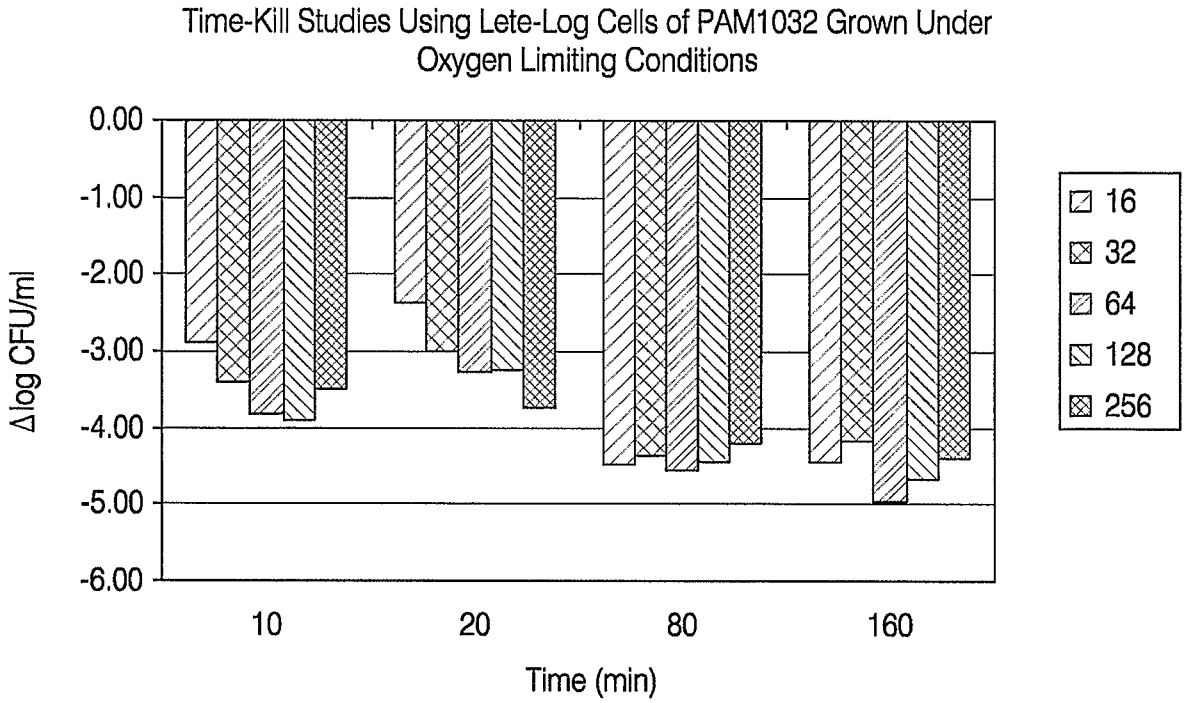

Time-Kill Studies Using Lete-Log Cells of PAM1032 Grown Under Oxygen Limiting Conditions
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
-6.00
Δlog CFU/ml
10
20
80
160
Time (min)
16
32
64
128
256

FIG. 7B

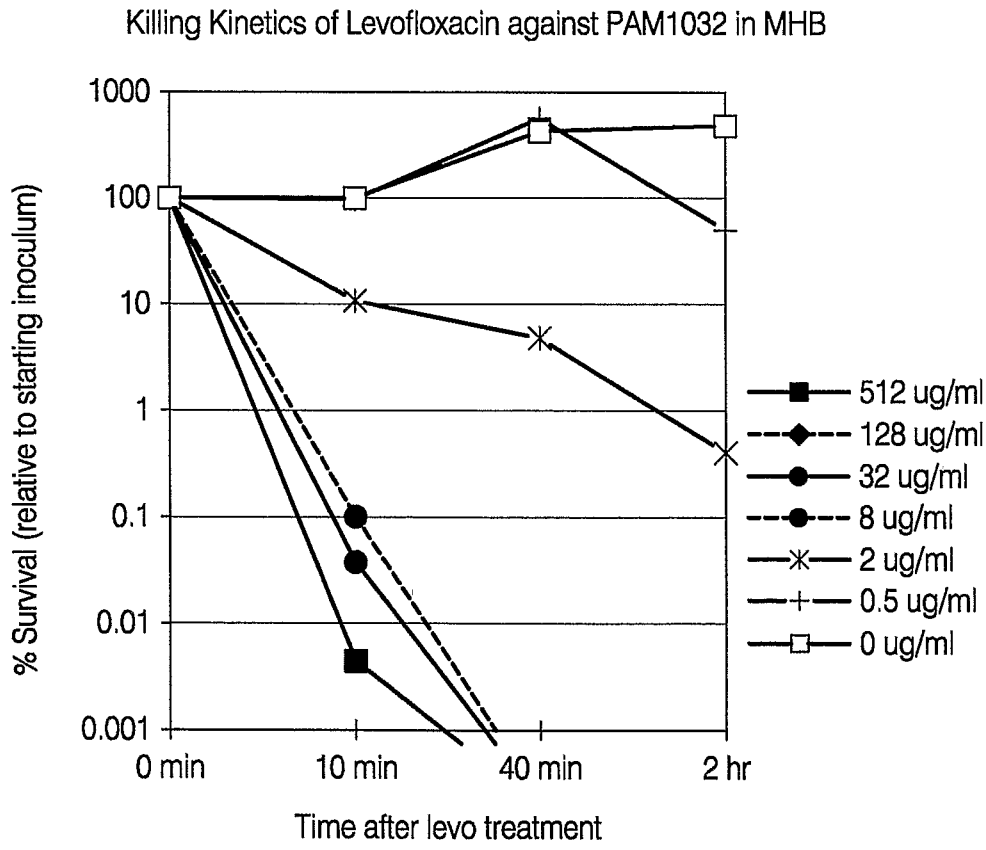

Killing Kinetics of Levofloxacin against PAM1032 in MHB
1000
100
10
1
0.1
0.01
0.001
% Survival (relative to starting inoculum)
0 min
10 min
40 min
2 hr
Time after levo treatment
512 ug/ml
128 ug/ml
32 ug/ml
8 ug/ml
2 ug/ml
0.5 ug/ml
0 ug/ml

FIG. 8A

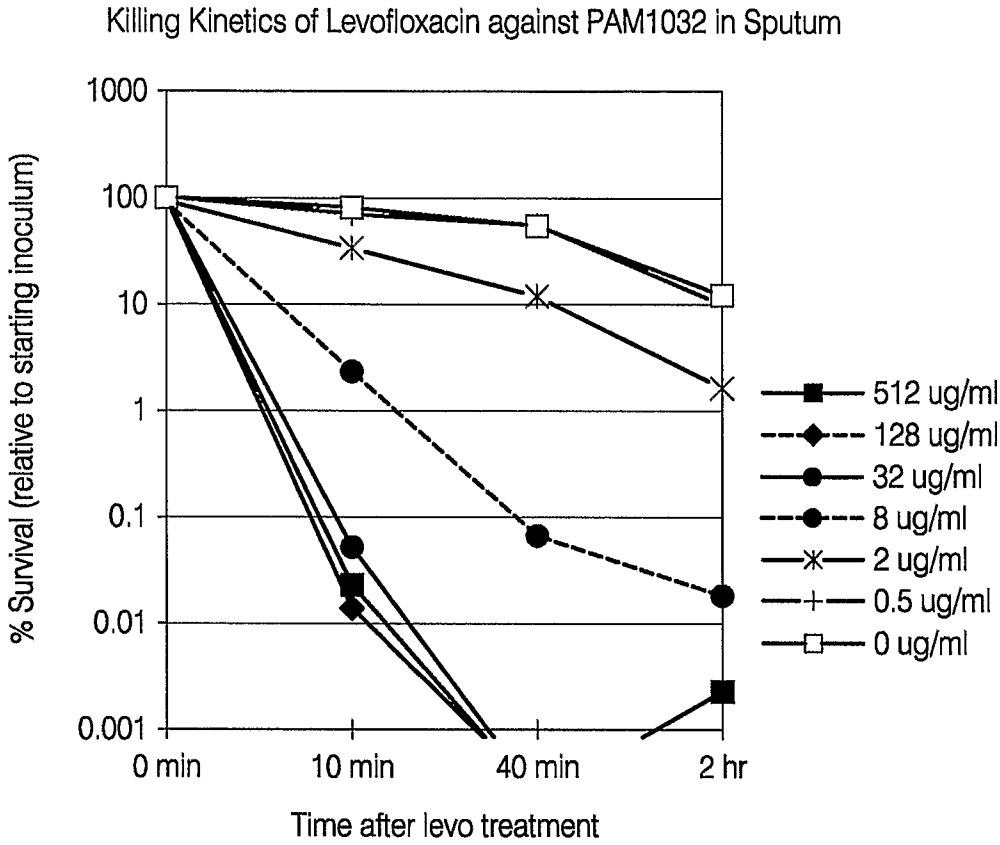

Killing Kinetics of Levofloxacin against PAM1032 in Sputum
% Survival (relative to starting inoculum)
1000
100
10
1
0.1
0.01
0.001
0 min
10 min
40 min
2 hr
Time after levo treatment
512 ug/ml
128 ug/ml
32 ug/ml
8 ug/ml
2 ug/ml
0.5 ug/ml
0 ug/ml

FIG. 8B

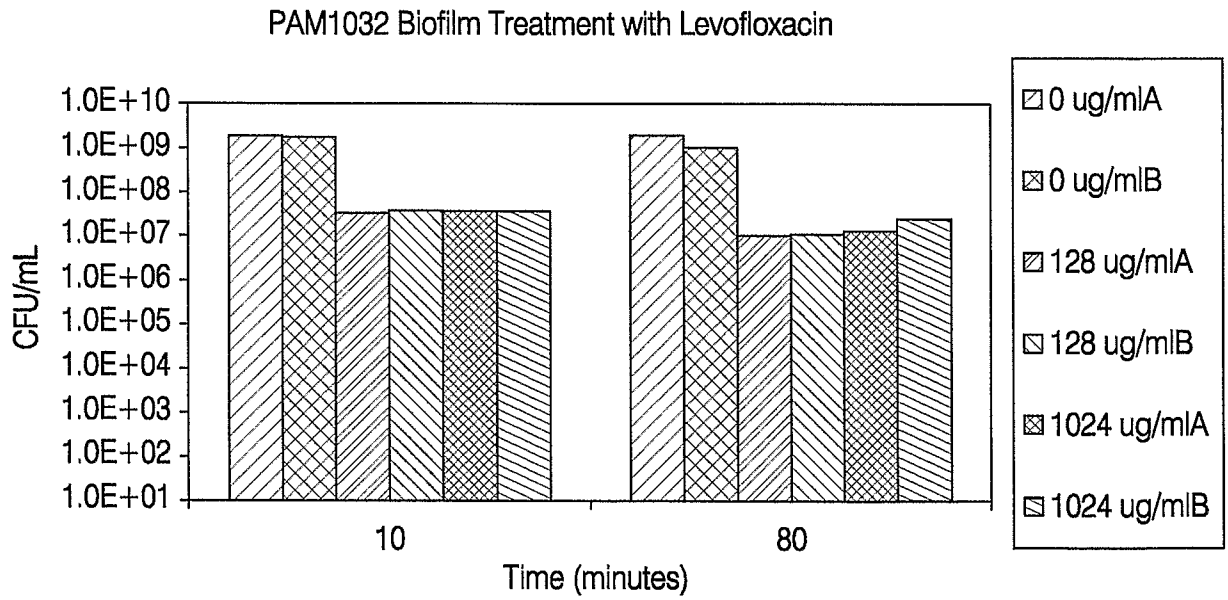

PAM1032 Biofilm Treatment with Levofloxacin
CFU/mL
1.0E+10
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
10
80
Time (minutes)
0 ug/mlA
0 ug/mlB
128 ug/mlA
128 ug/mlB
1024 ug/mlA
1024 ug/mlB

FIG. 9

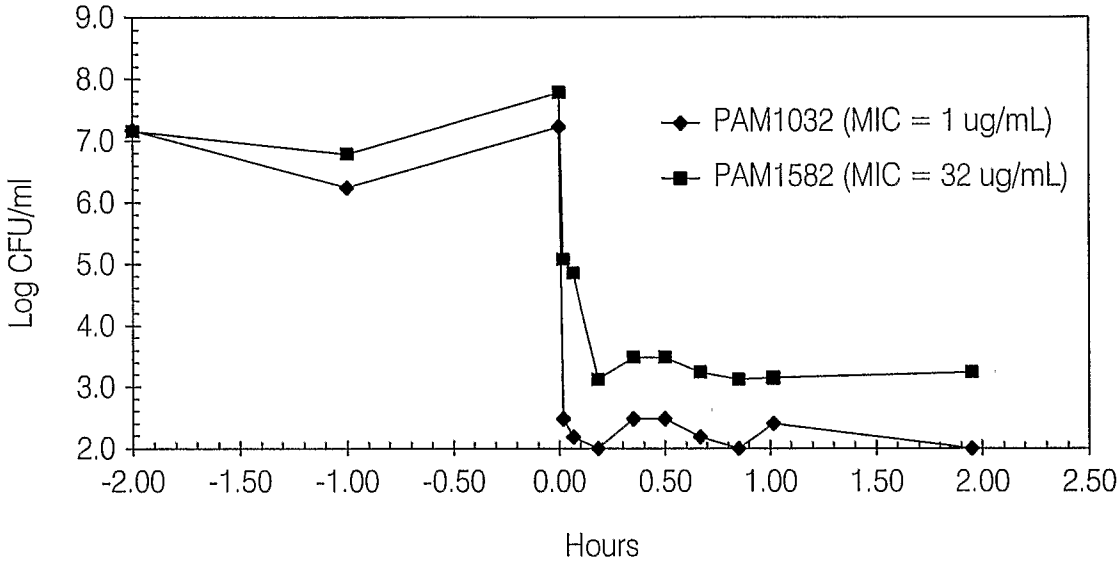

9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
Log CFU/ml
-2.00
-1.50
-1.00
-0.50
0.00
0.50
1.00
1.50
2.00
2.50
Hours
PAM1032 (MIC = 1 ug/mL)
PAM1582 (MIC = 32 ug/mL)

FIG. 10

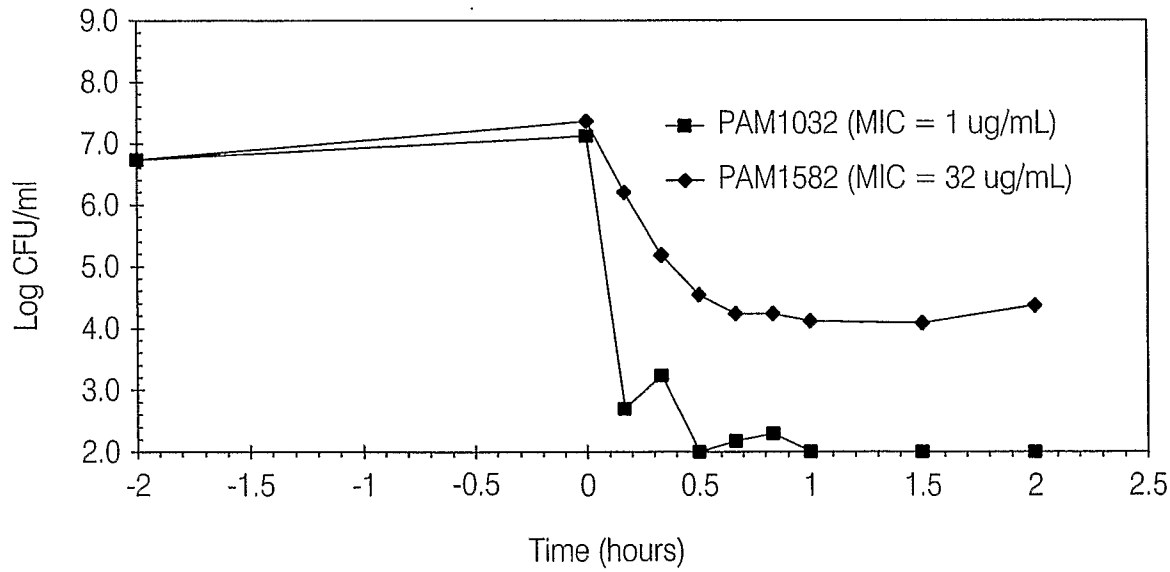

9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
Log CFU/ml
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2
2.5
Time (hours)
PAM1032 (MIC = 1 ug/mL)
PAM1582 (MIC = 32 ug/mL)

FIG. 11

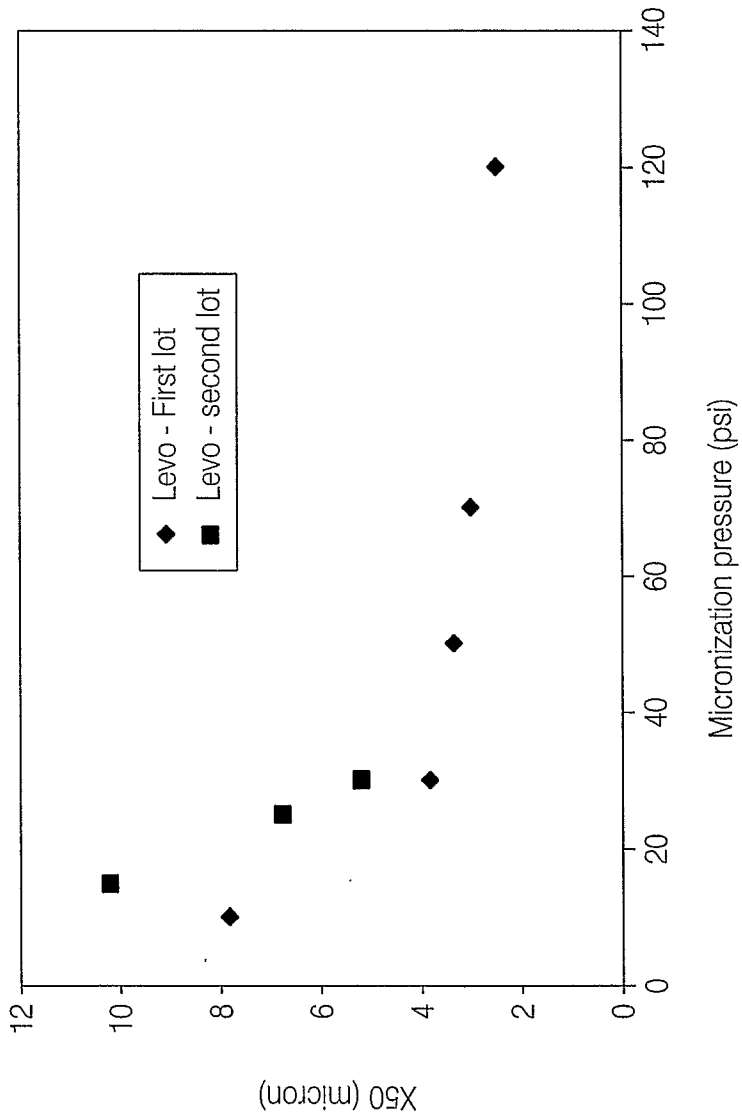

Levo - First lot
Levo - second lot
X50 (micron)
Micronization pressure (psi)
0
2
4
6
8
10
12
0
20
40
60
80
100
120
140

FIG. 12

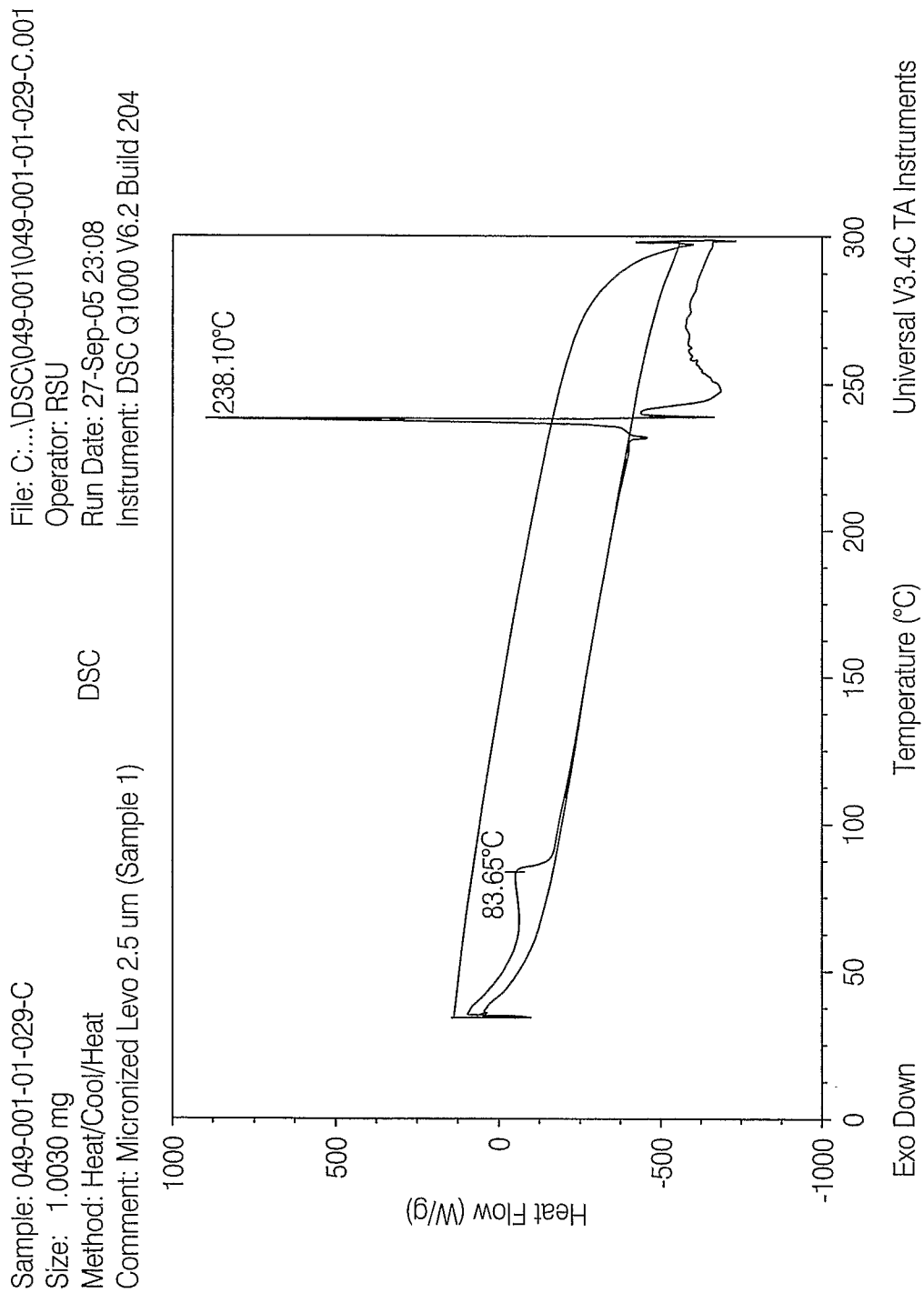

Sample: 049-001-01-029-C
Size: 1.0030 mg
Method: Heat/Cool/Heat
Comment: Micronized Levo 2.5 um (Sample 1)
DSC
File: C:...\DSC\049-001\049-001-01-029-C.001
Operator: RSU
Run Date: 27-Sep-05 23:08
Instrument: DSC Q1000 V6.2 Build 204
238.10°C
83.65°C
Heat Flow (W/g)
1000
500
0
-500
-1000
0
50
100
150
200
250
300
Temperature (°C)
Exo Down
Universal V3.4C TA Instruments

FIG. 13

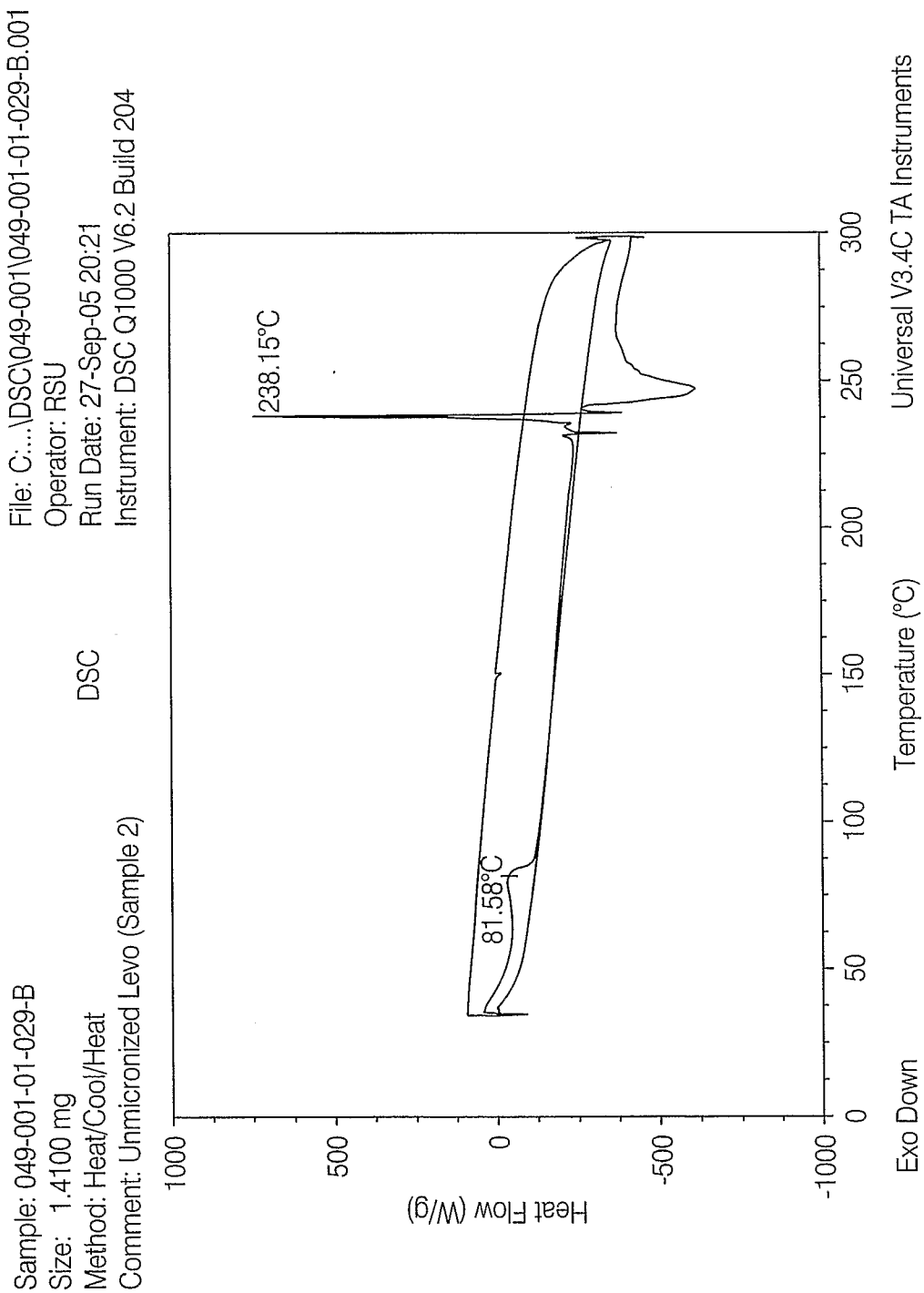
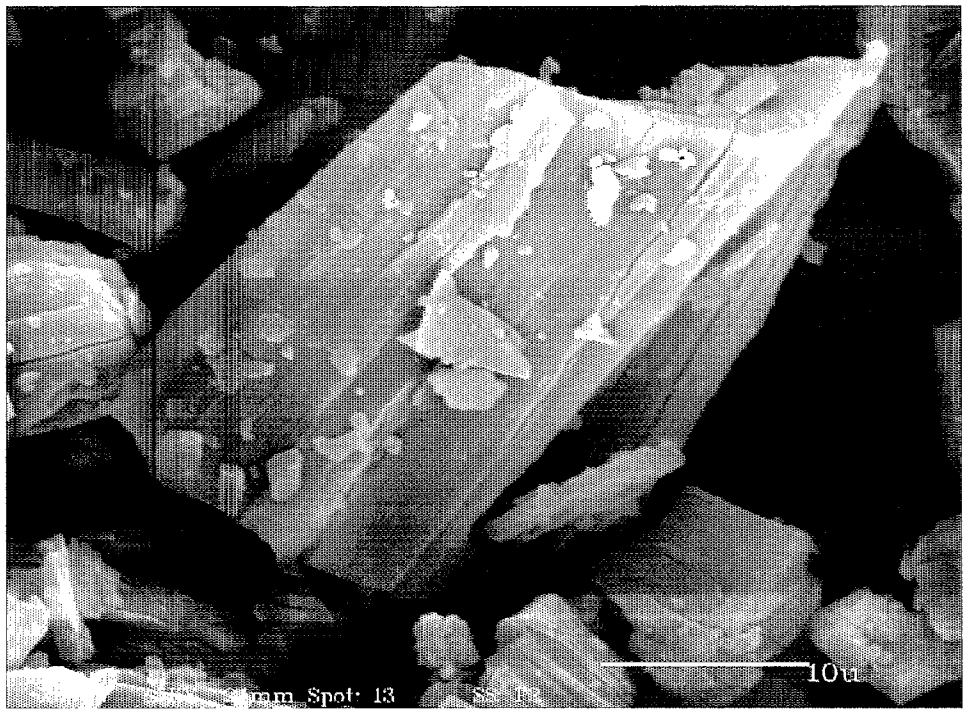


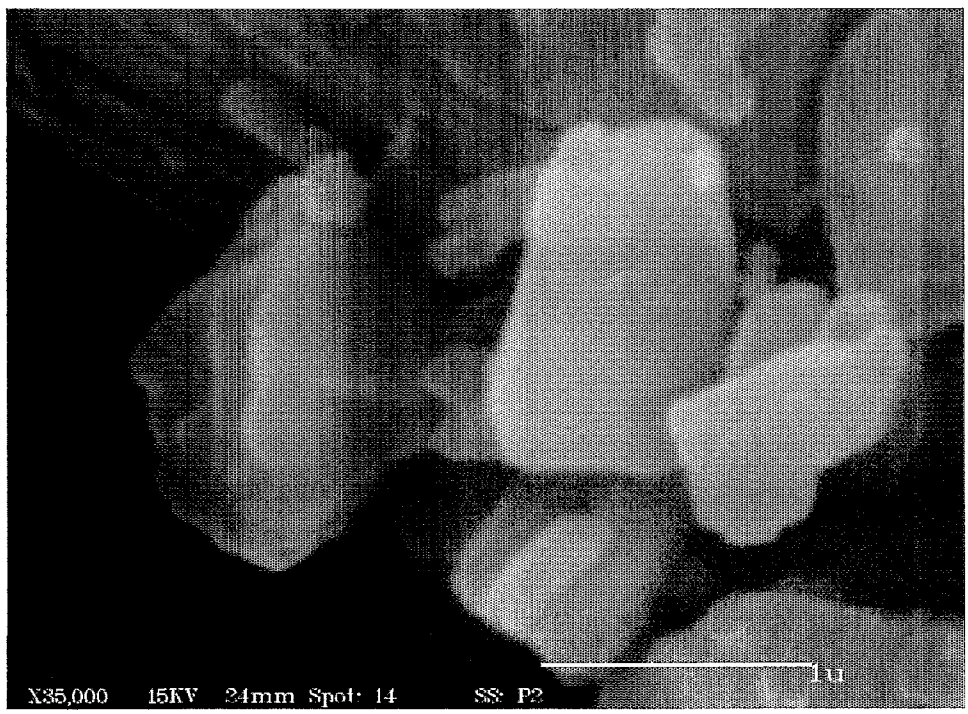
FIG. 13 continued



Title: Unmicronized
Comment:

Date: 09-28-2005 Time: 10:38
Filename: TEMP.TIF

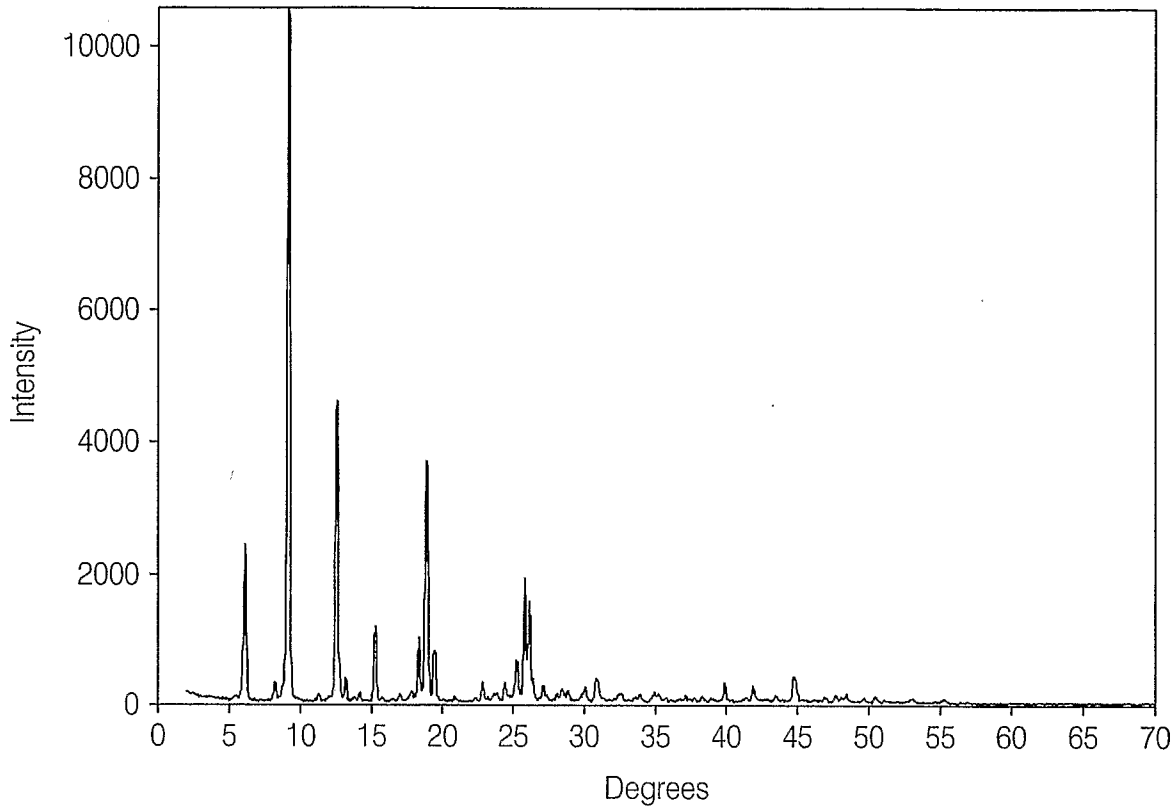
FIG. 14A



Title: MICRONIZED2.5
Comment:

Date: 09-28-2005 Time: 11:08
Filename: TEMP.TIF

FIG. 14B


10000
8000
6000
4000
2000
0
Intensity
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70
Degrees

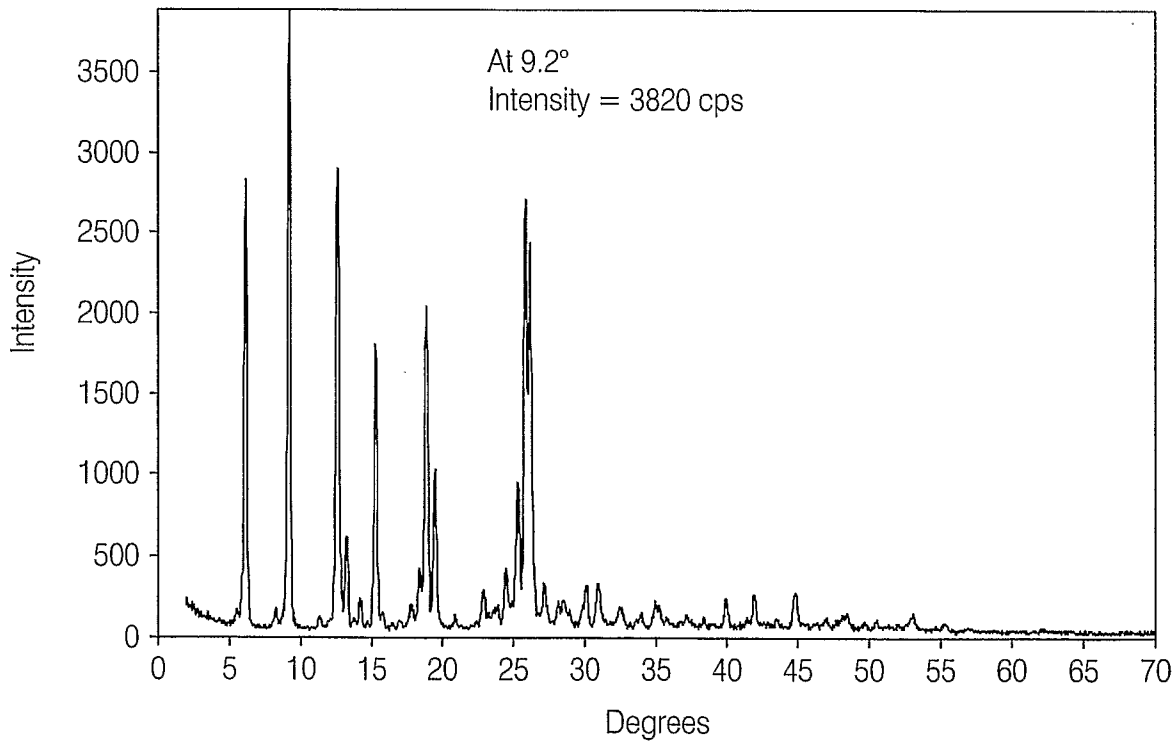

At 9.2°
Intensity = 3820 cps
3500
3000
2500
2000
1500
1000
500
0
Intensity
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70
Degrees

FIG. 15

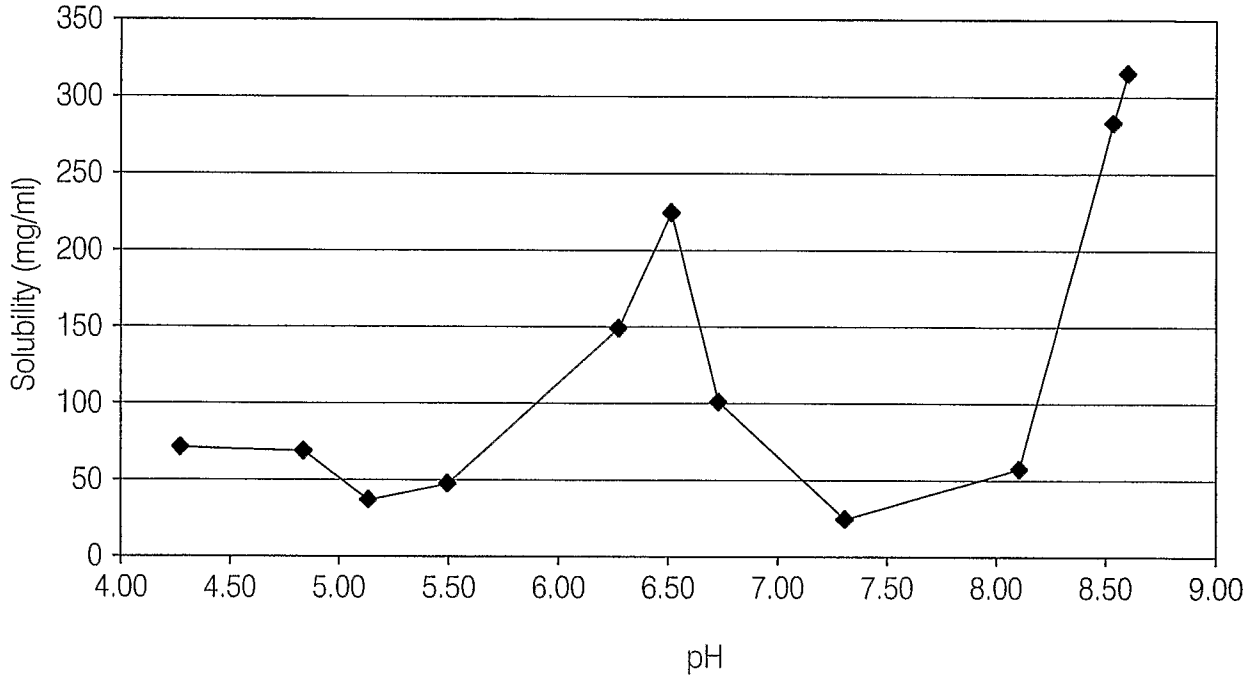

350
300
250
200
150
100
50
0
Solubility (mg/ml)
4.00
4.50
5.00
5.50
6.00
6.50
7.00
7.50
8.00
8.50
9.00
pH

FIG. 16

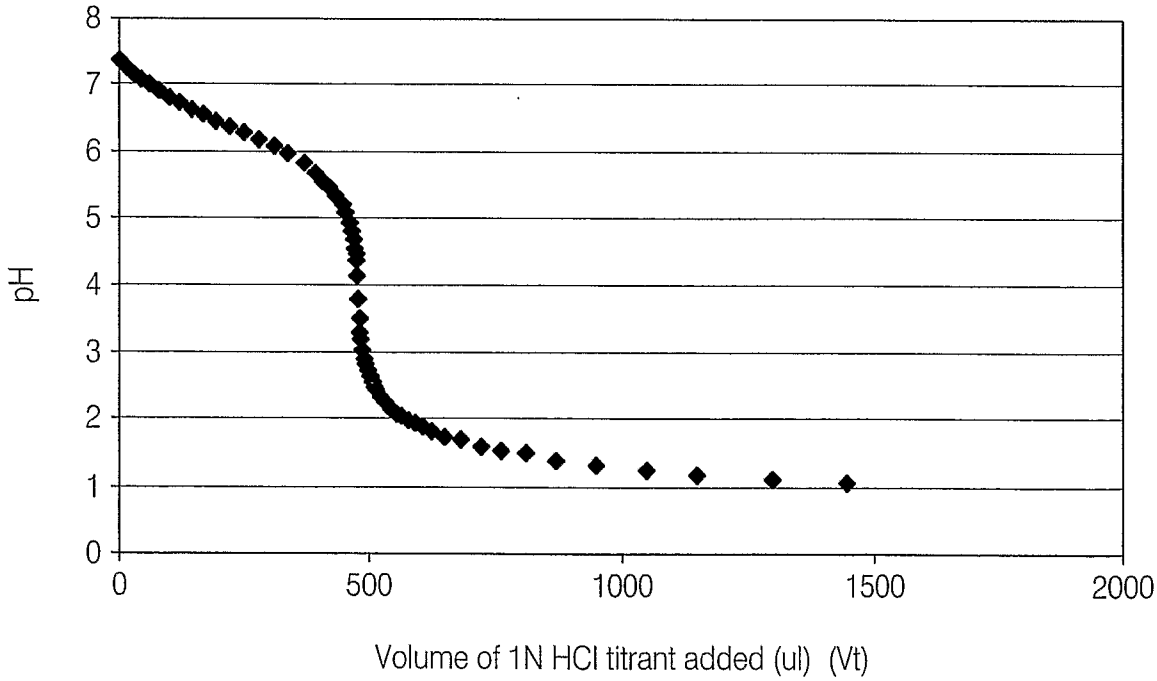

8
7
6
5
4
3
2
1
0
pH
0
500
1000
1500
2000
Volume of 1N HCl titrant added (ul) (Vt)

FIG. 17

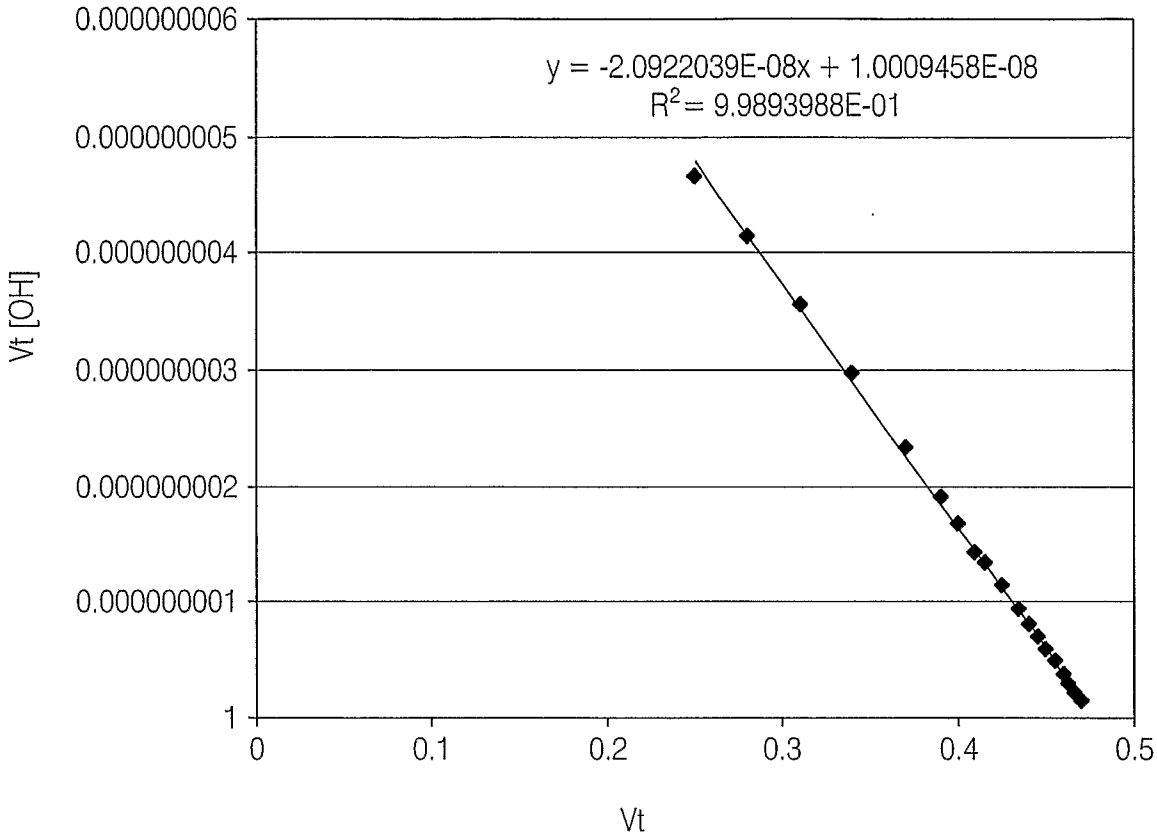

y = -2.0922039E-08x + 1.0009458E-08
R2= 9.9893988E-01
Vt [OH]
0.000000006
0.000000005
0.000000004
0.000000003
0.000000002
0.000000001
1
0
0.1
0.2
0.3
0.4
0.5
Vt

FIG. 18

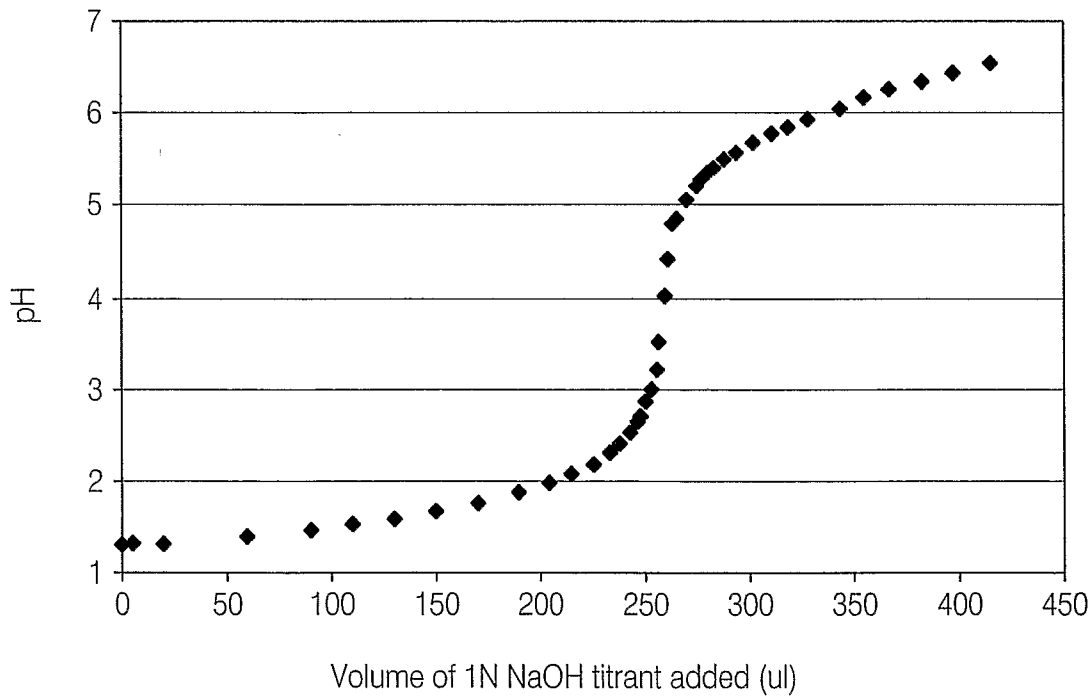

pH
7
6
5
4
3
2
1
0
50
100
150
200
250
300
350
400
450
Volume of 1N NaOH titrant added (ul)

FIG. 19

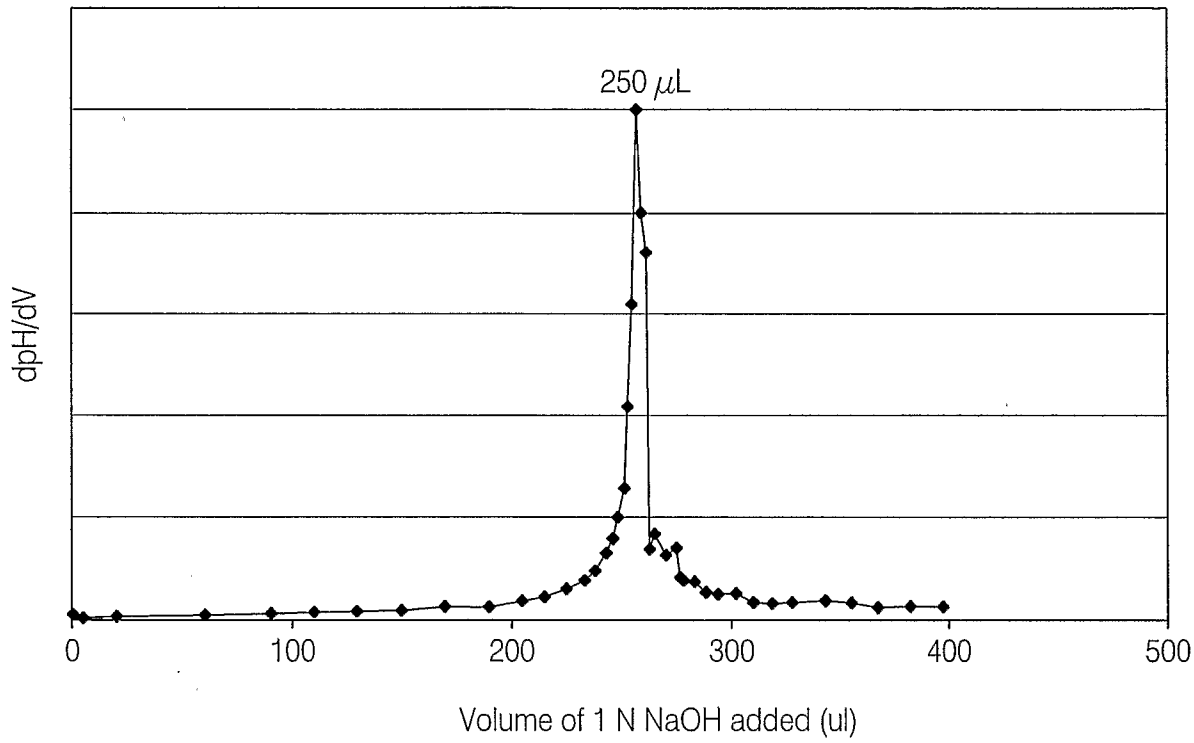

250 μL
dpH/dV
0
100
200
300
400
500
Volume of 1 N NaOH added (ul)

FIG. 20

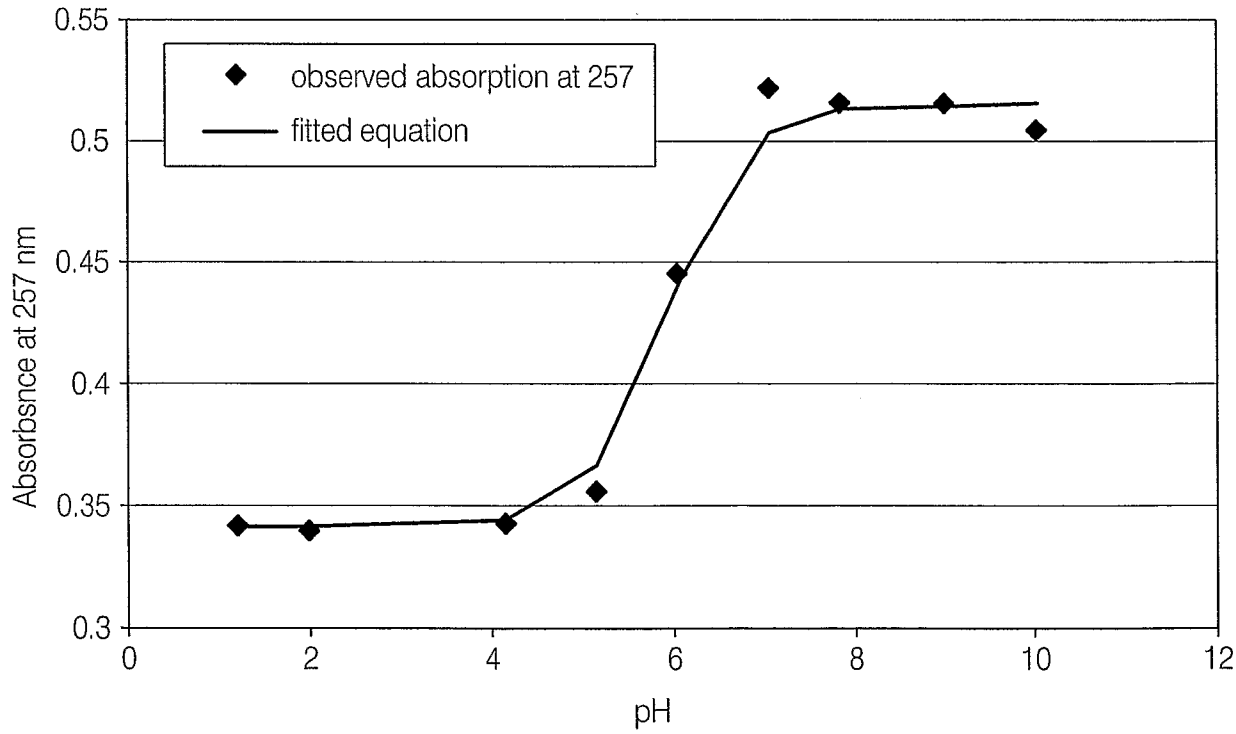

observed absorption at 257
fitted equation
Absorbsnce at 257 nm
0.55
0.5
0.45
0.4
0.35
0.3
0
2
4
6
8
10
12
pH

FIG. 21

a) Pamoic acid
mp = 330.1°C

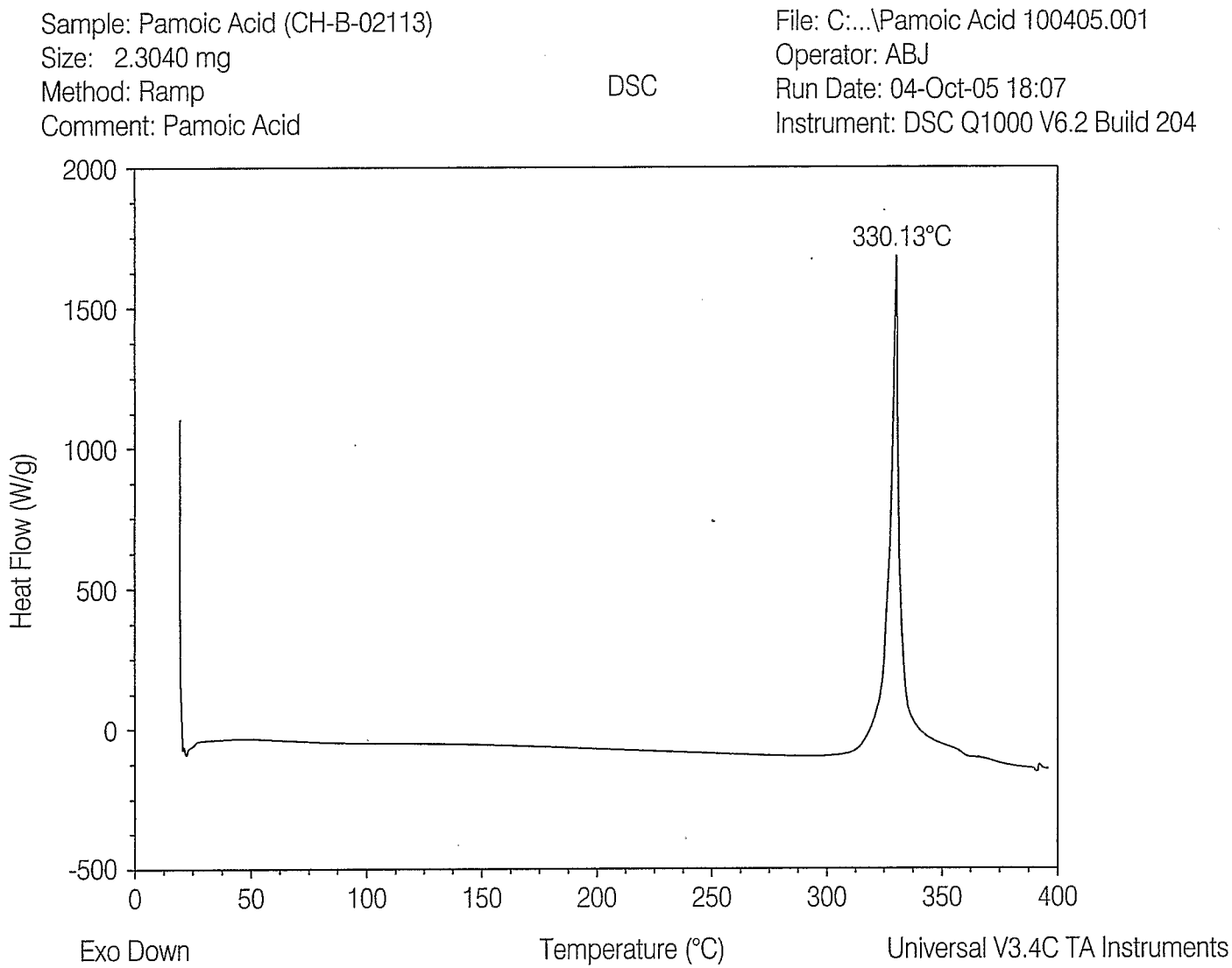


FIG. 22

b) Levofloxacin base
mp = 239.2°C

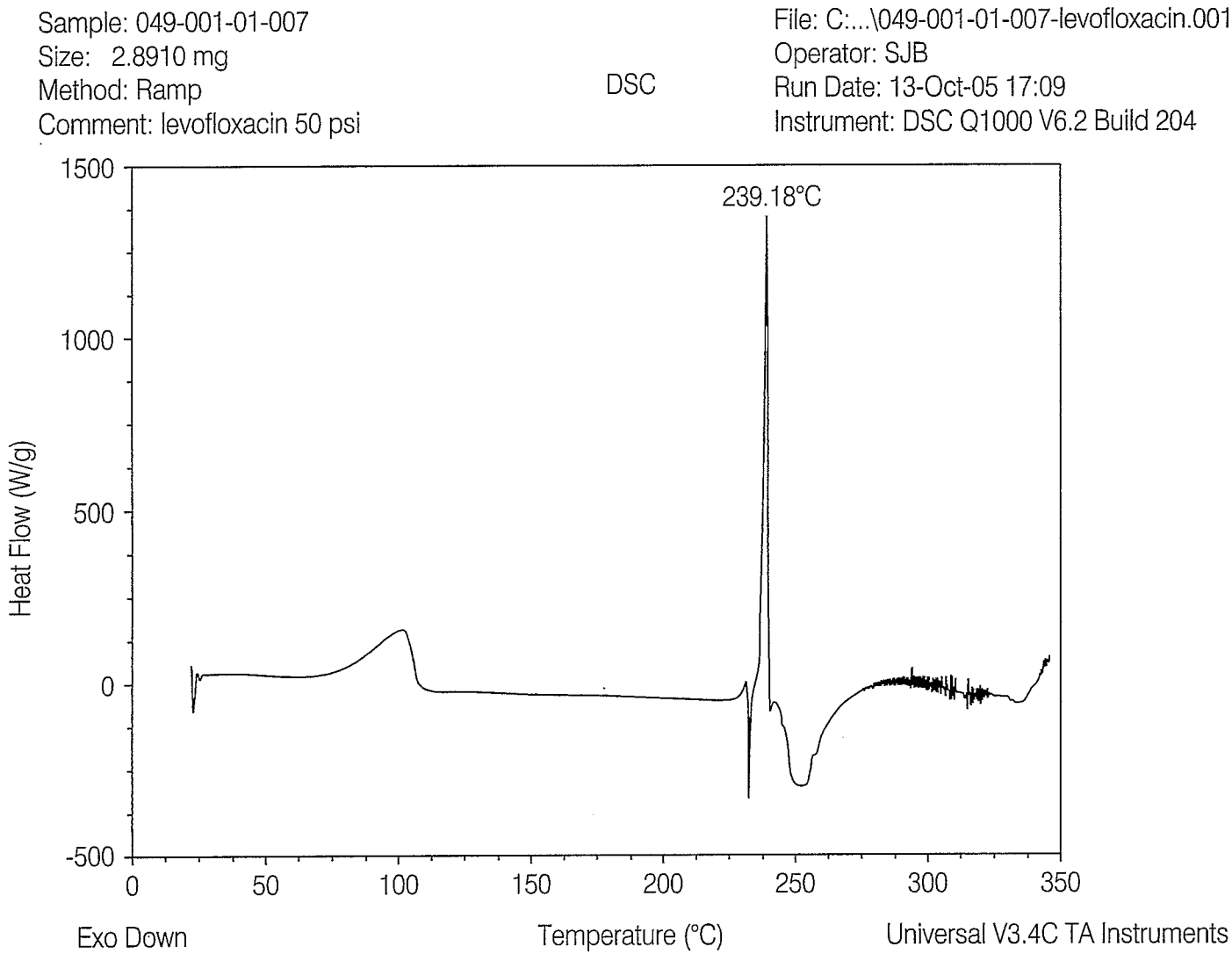


FIG. 22 continued

c) Levofloxacin pamoic acid co-crystals
mp = 210.6°C

Sample: 049-001-03-011 dry
Size: 2.3430 mg
Method: Ramp
Comment: levofloxacin pamoate "salt"

DSC

File: C:...\049-001-03-011 dry.001
Operator: SJB
Run Date: 13-Oct-05 15:50
Instrument: DSC Q1000 V6.2 Build 204

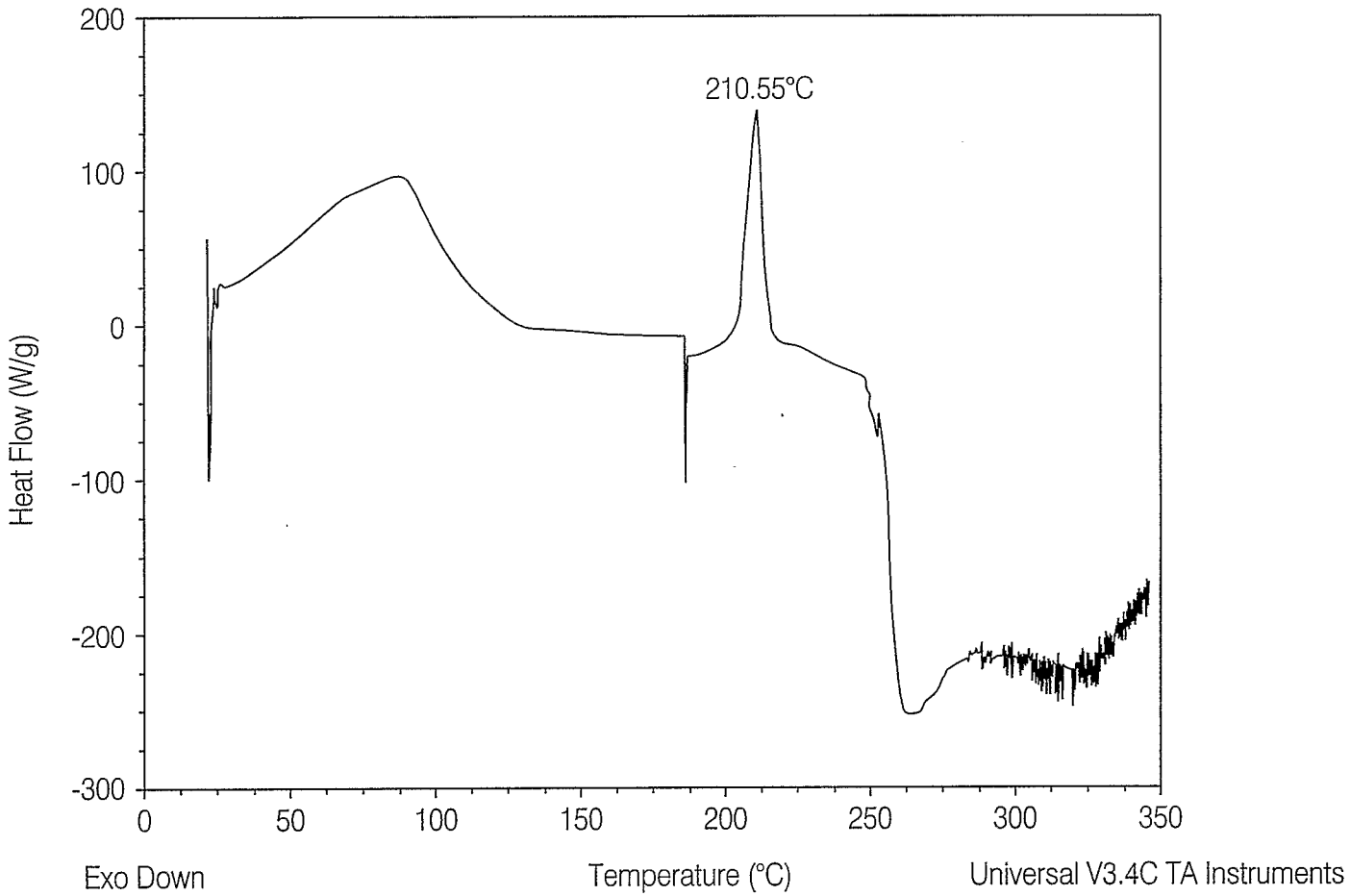


FIG. 22 continued

d) 1:1 levo-pamoate mixture

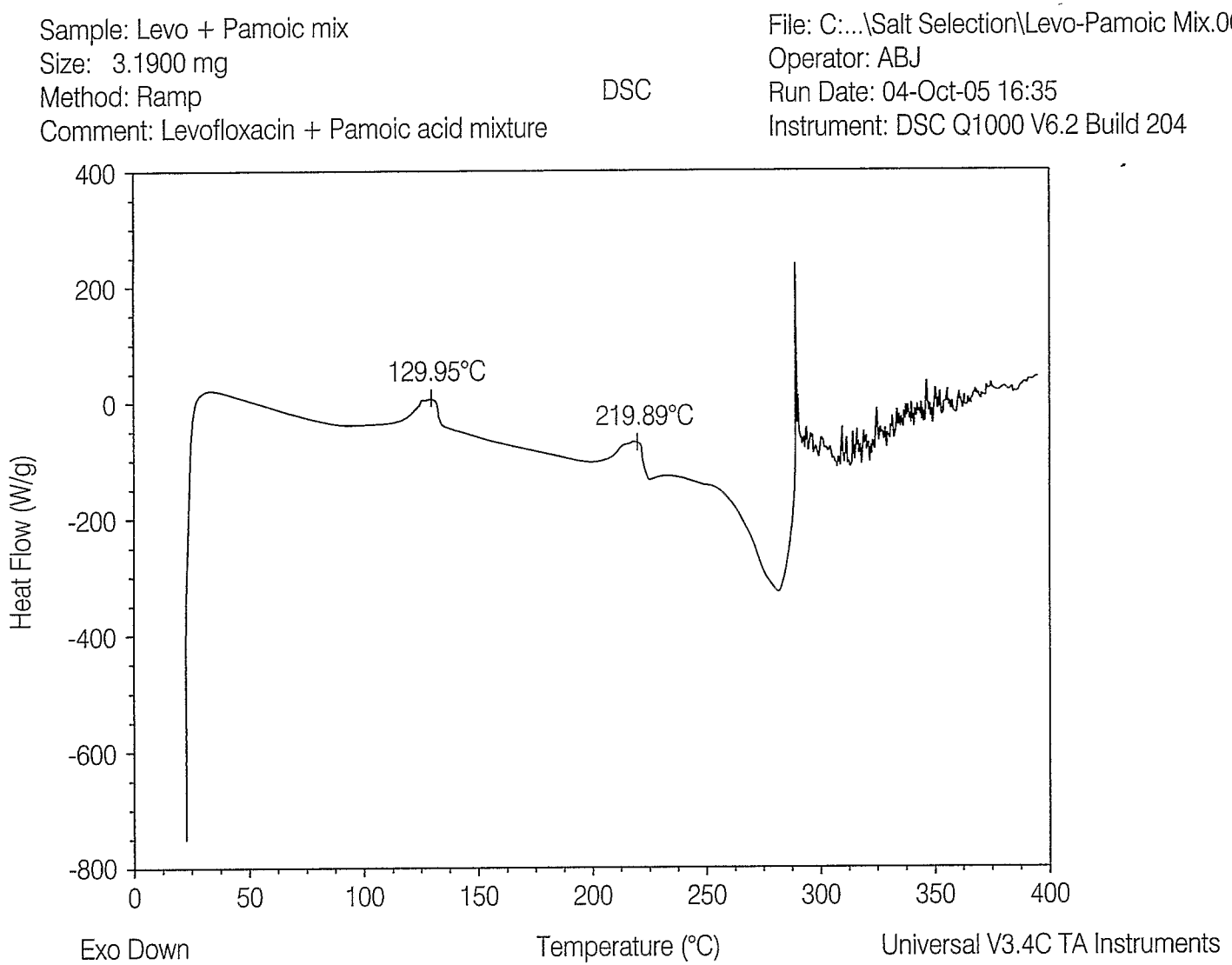


FIG. 22 continued

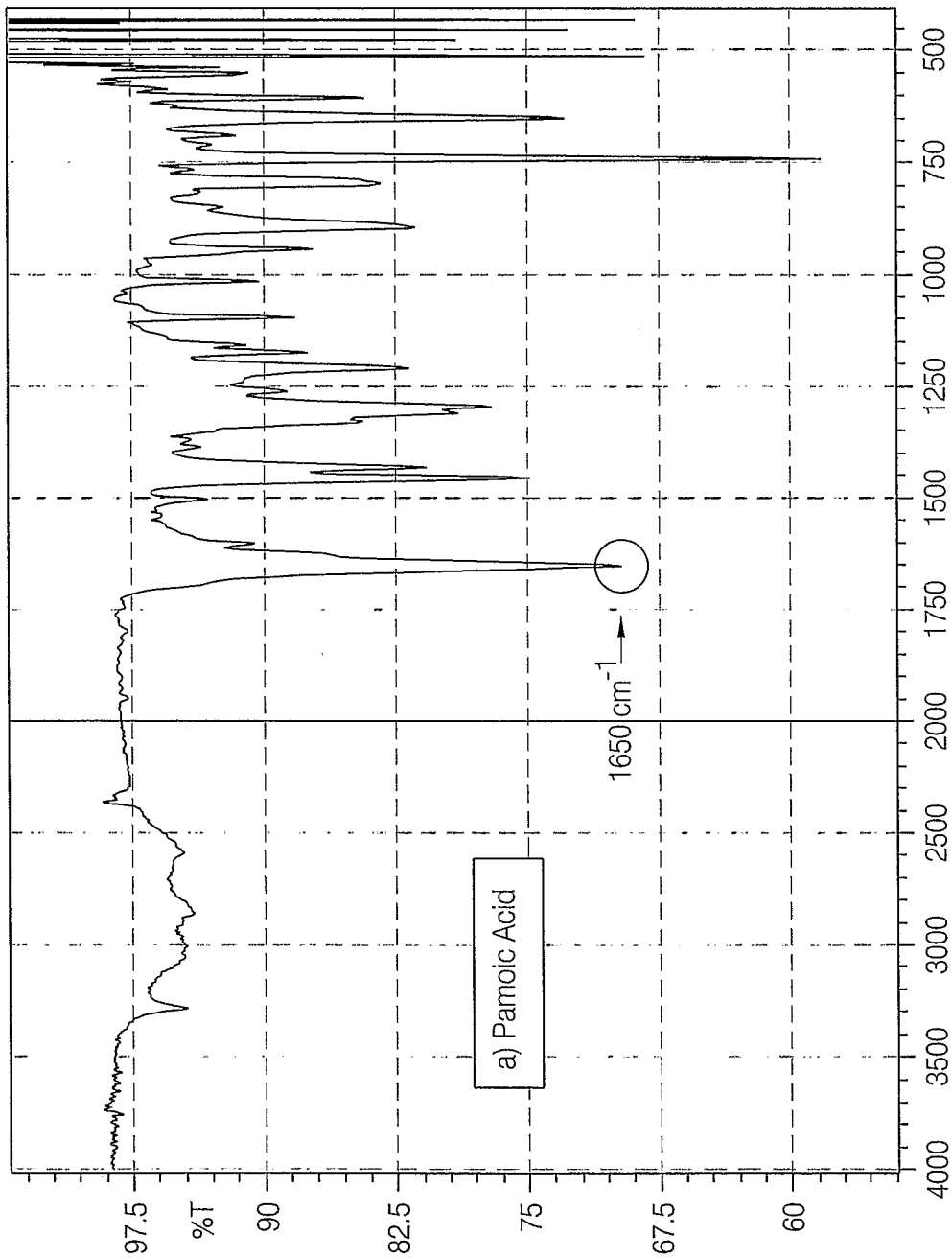

a) Pamoic Acid
1650 cm-1
%T
97.5
90
82.5
75
67.5
60
4000
3500
3000
2500
2000
1750
1500
1250
1000
750
500
1/cm

FIG. 23

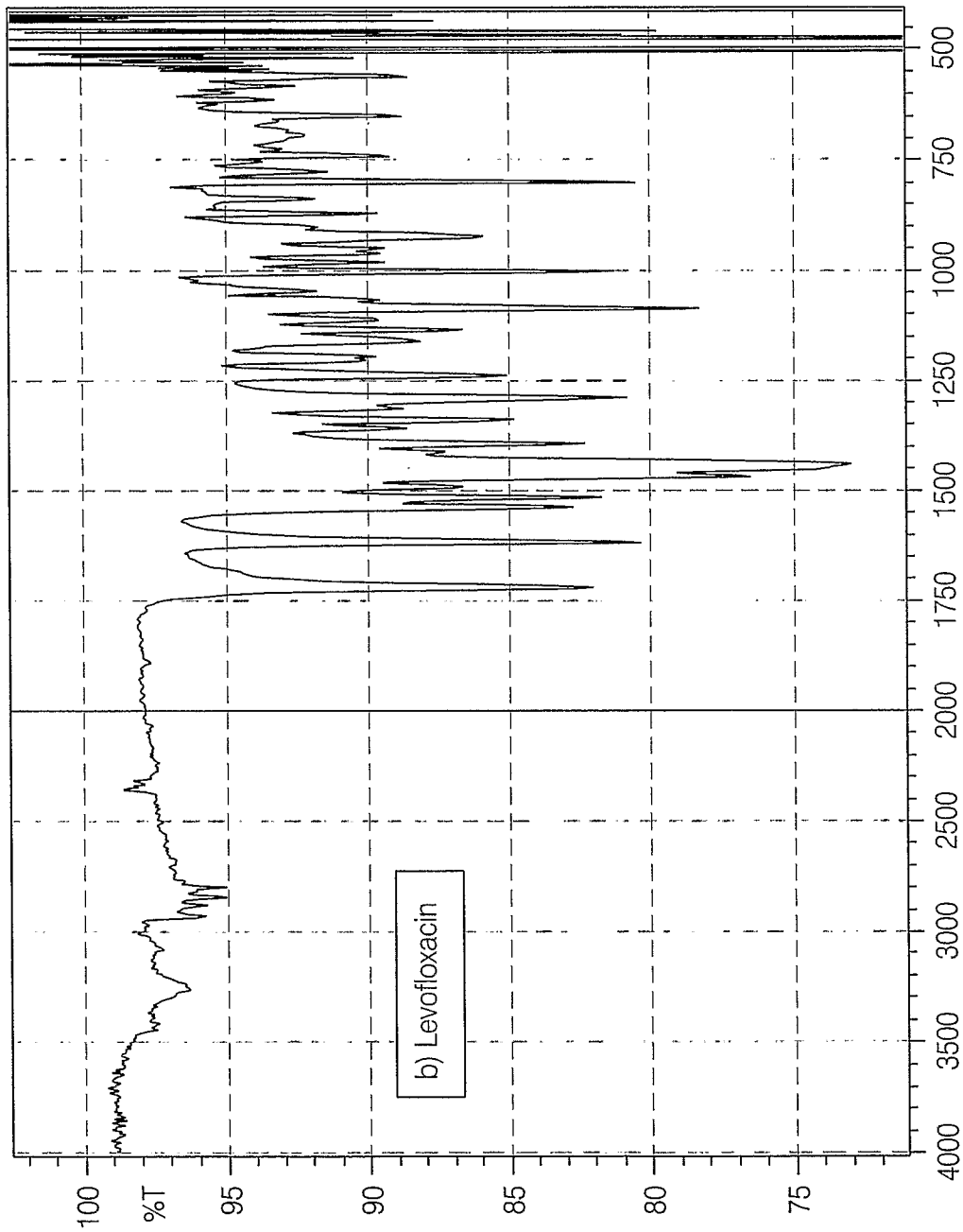


FIG. 23 continued

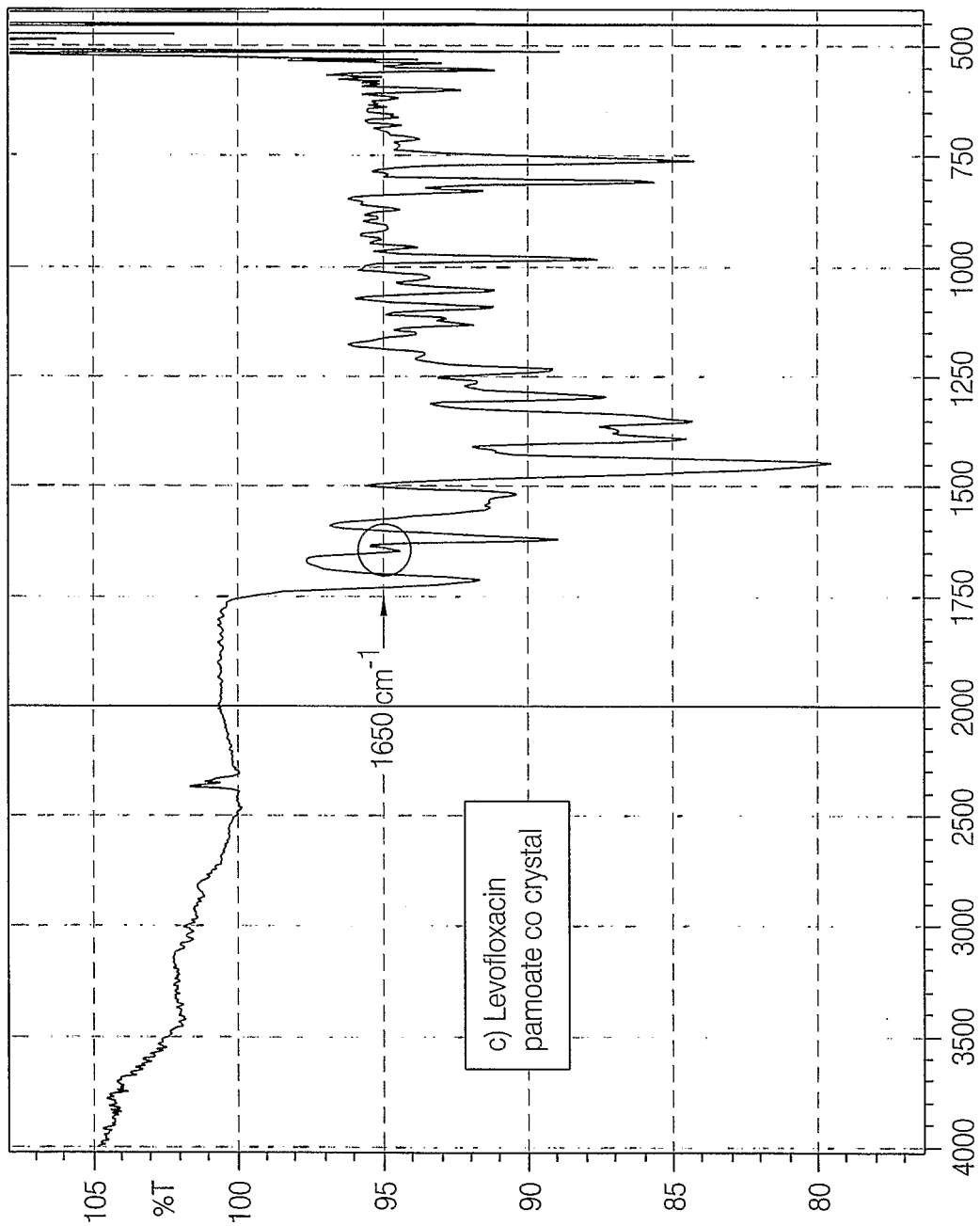


FIG. 23 continued

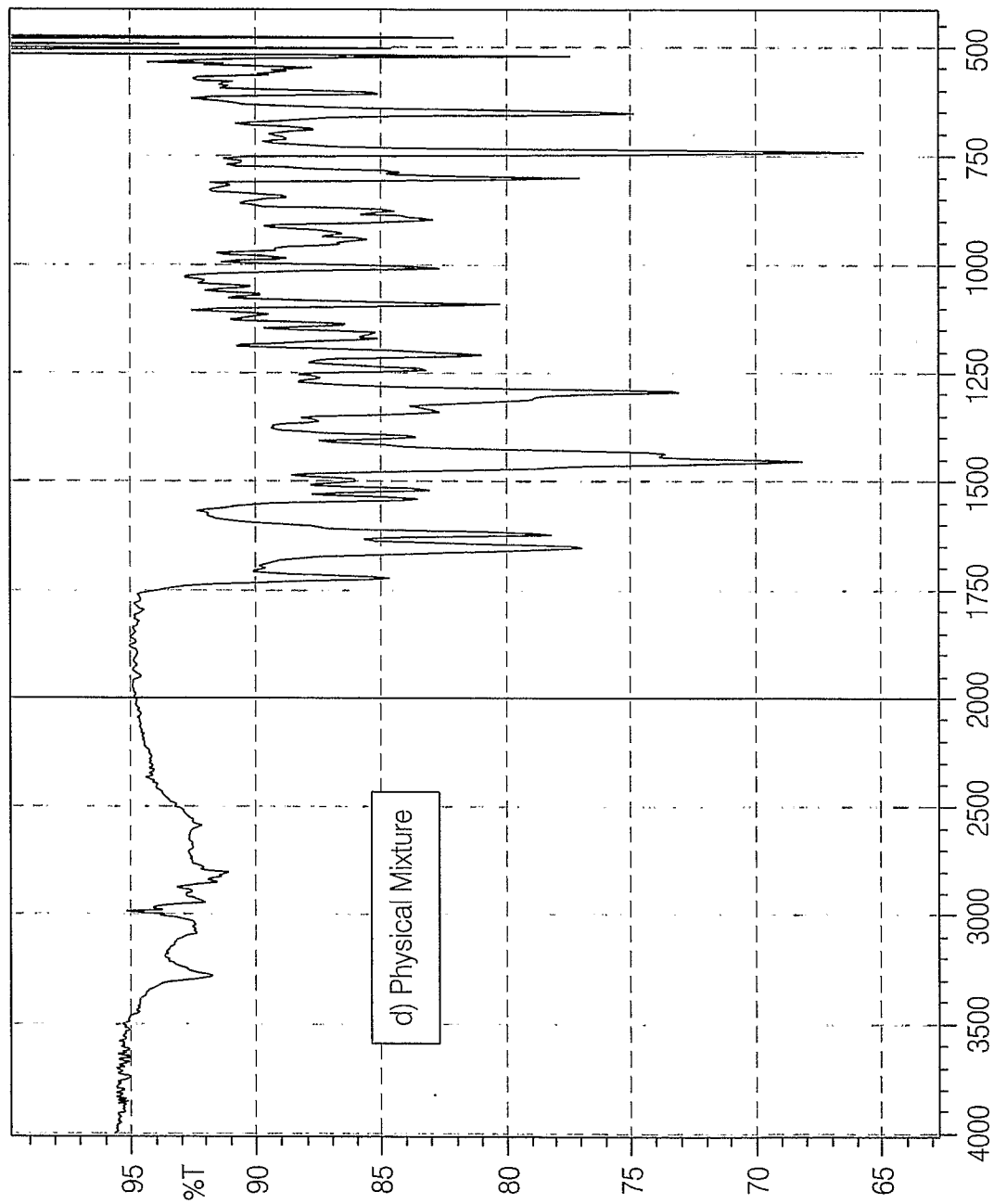


FIG. 23 continued

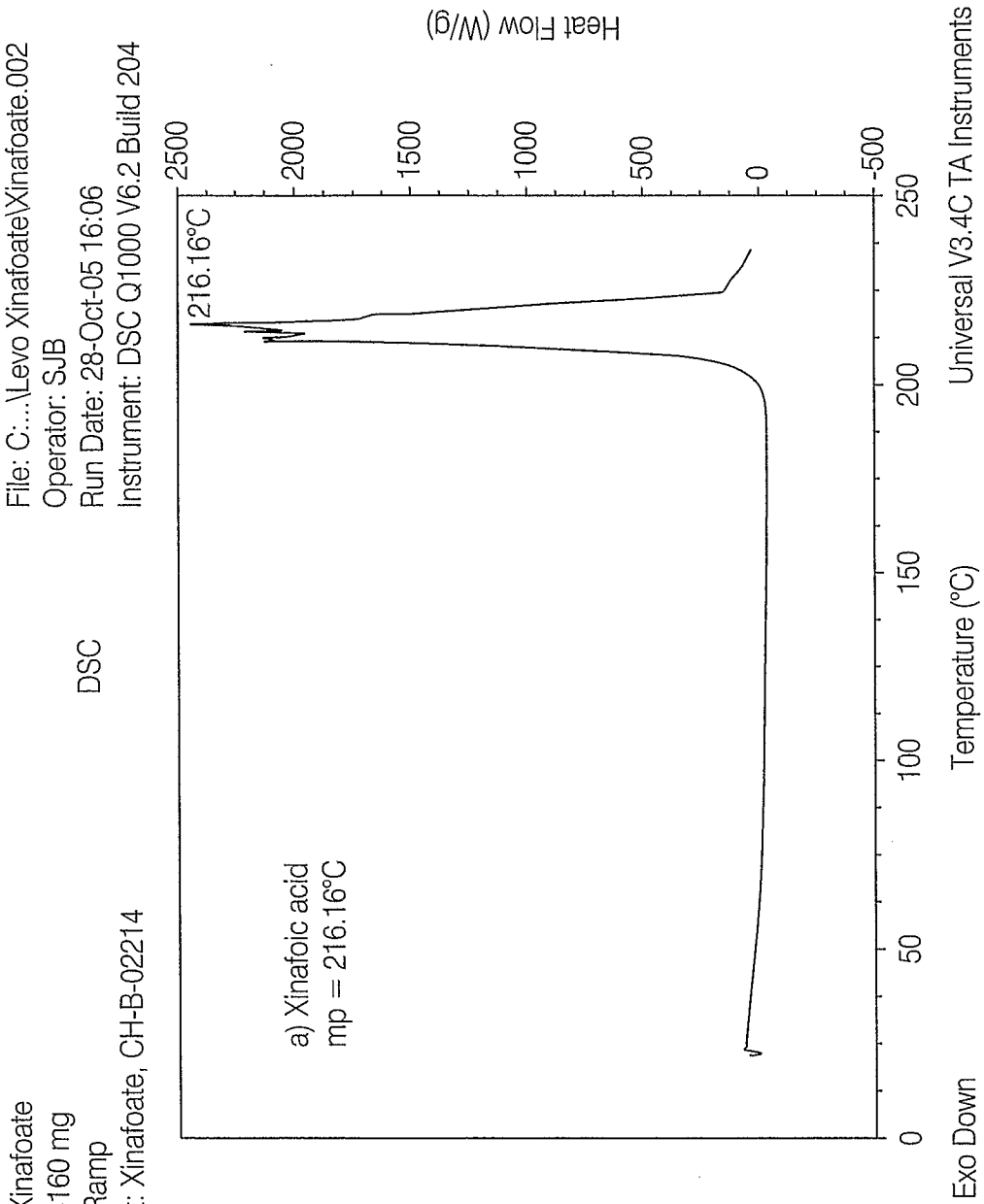

Sample: Xinafoate
Size: 3.4160 mg
Method: Ramp
Comment: Xinafoate, CH-B-02214
DSC
File: C:...\Levo Xinafoate\Xinafoate.002
Operator: SJB
Run Date: 28-Oct-05 16:06
Instrument: DSC Q1000 V6.2 Build 204
a) Xinafoic acid
mp = 216.16°C
216.16°C
Heat Flow (W/g)
2500
2000
1500
1000
500
0
-500
0
50
100
150
200
250
Temperature (°C)
Exo Down
Universal V3.4C TA Instruments

FIG. 24

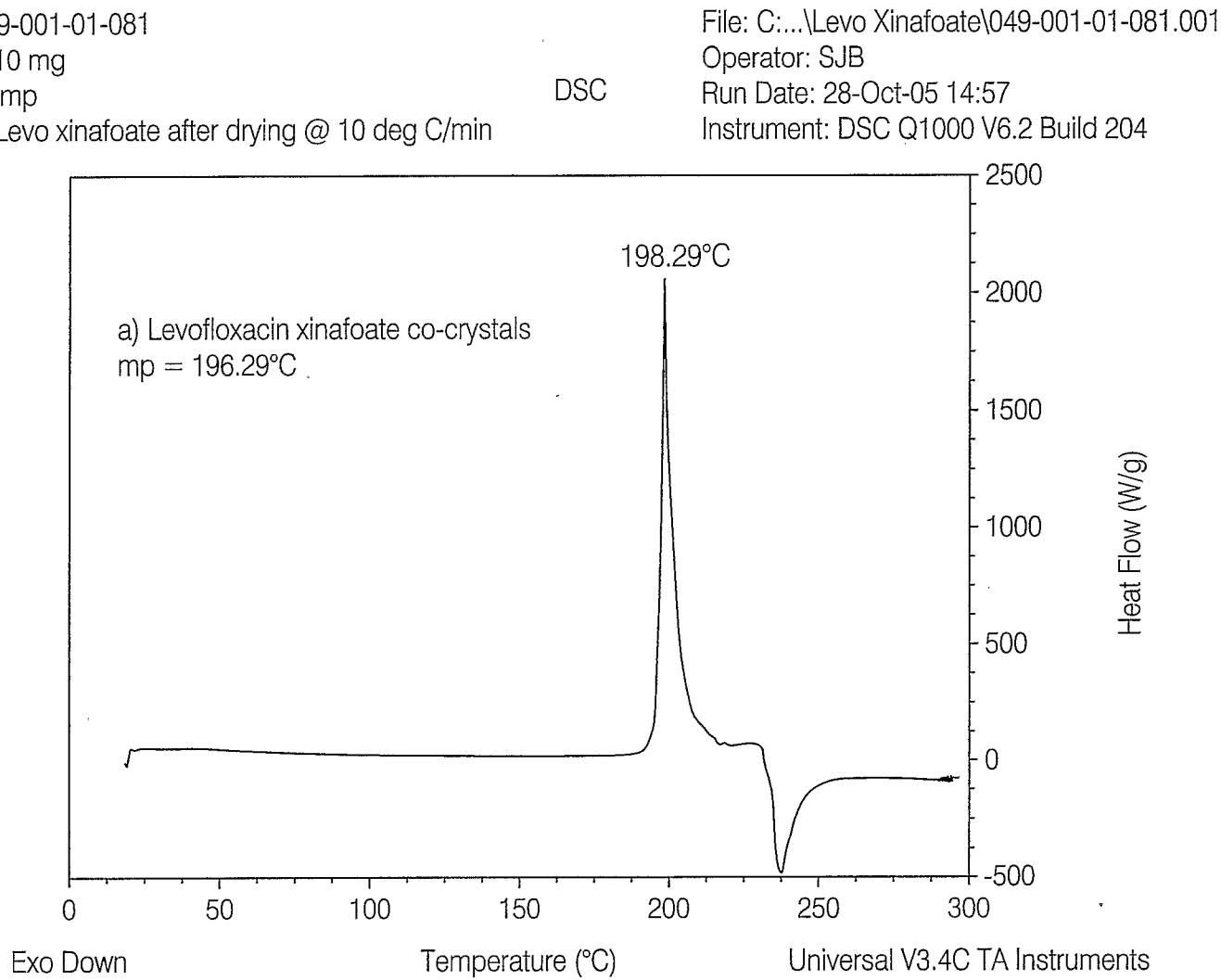


FIG. 24 continued

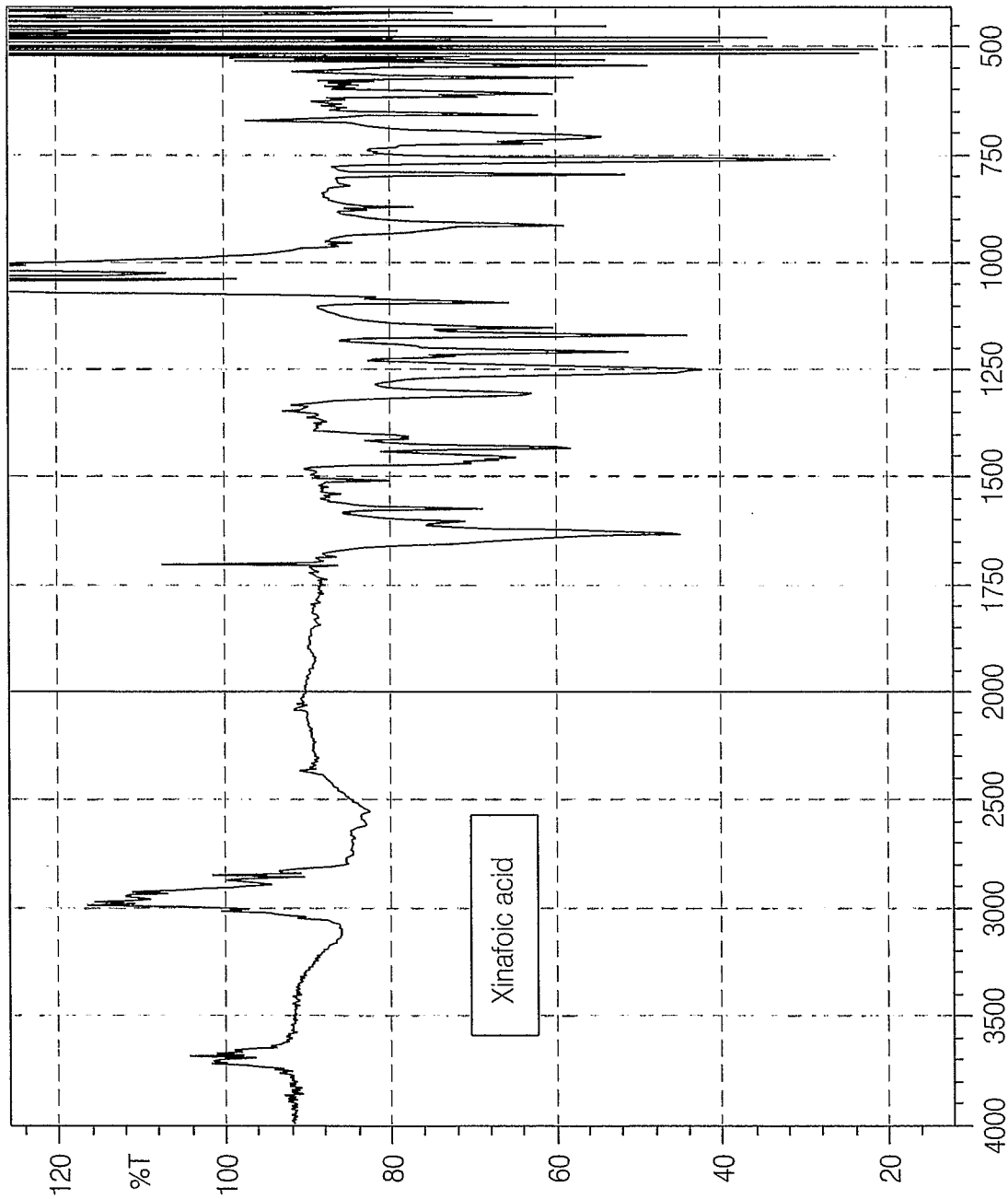

120
%T
100
80
60
40
20
4000
3500
3000
2500
2000
1750
1500
1250
1000
750
500
1/cm
Xinafoic acid

FIG. 25

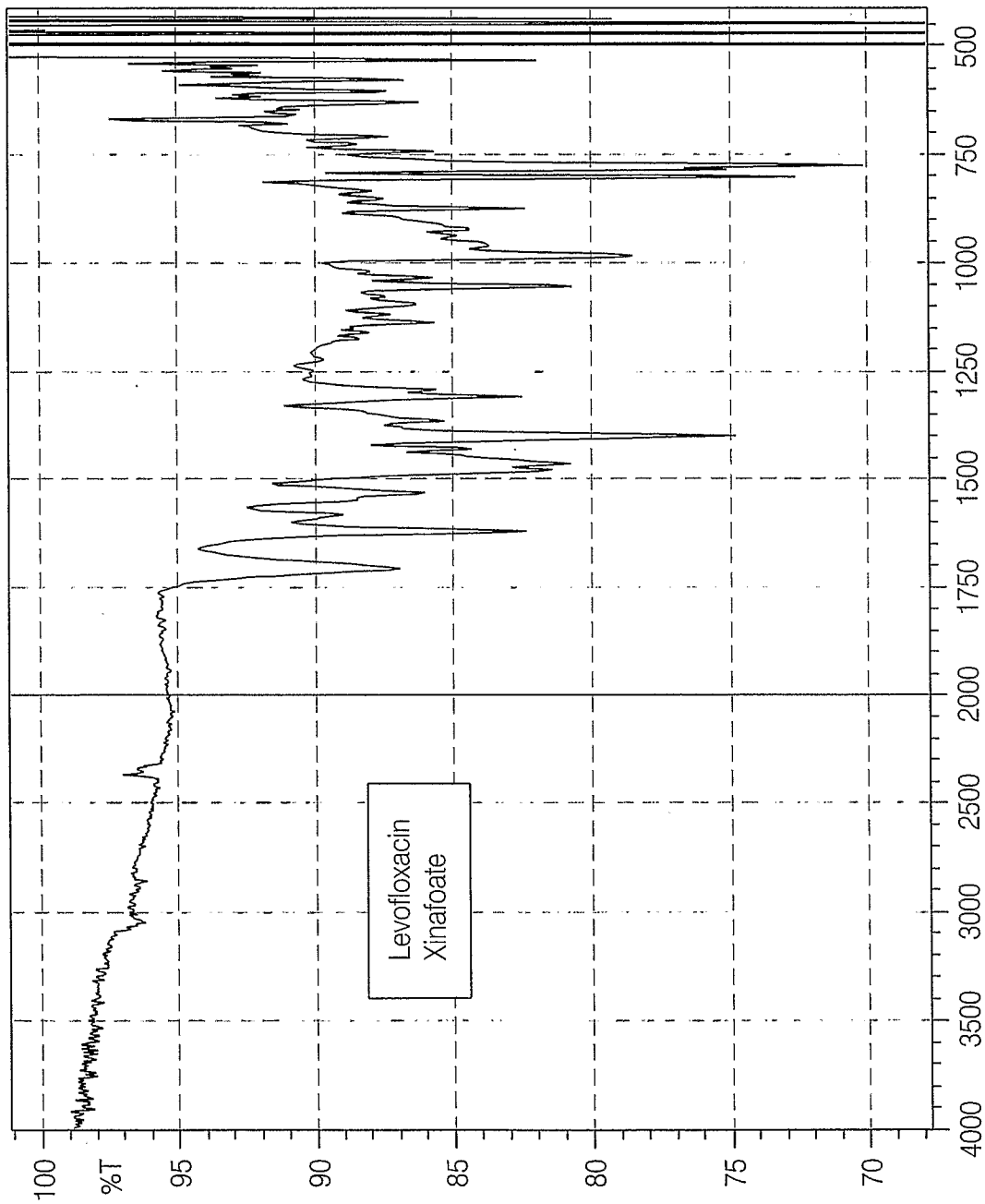


FIG. 25 continued

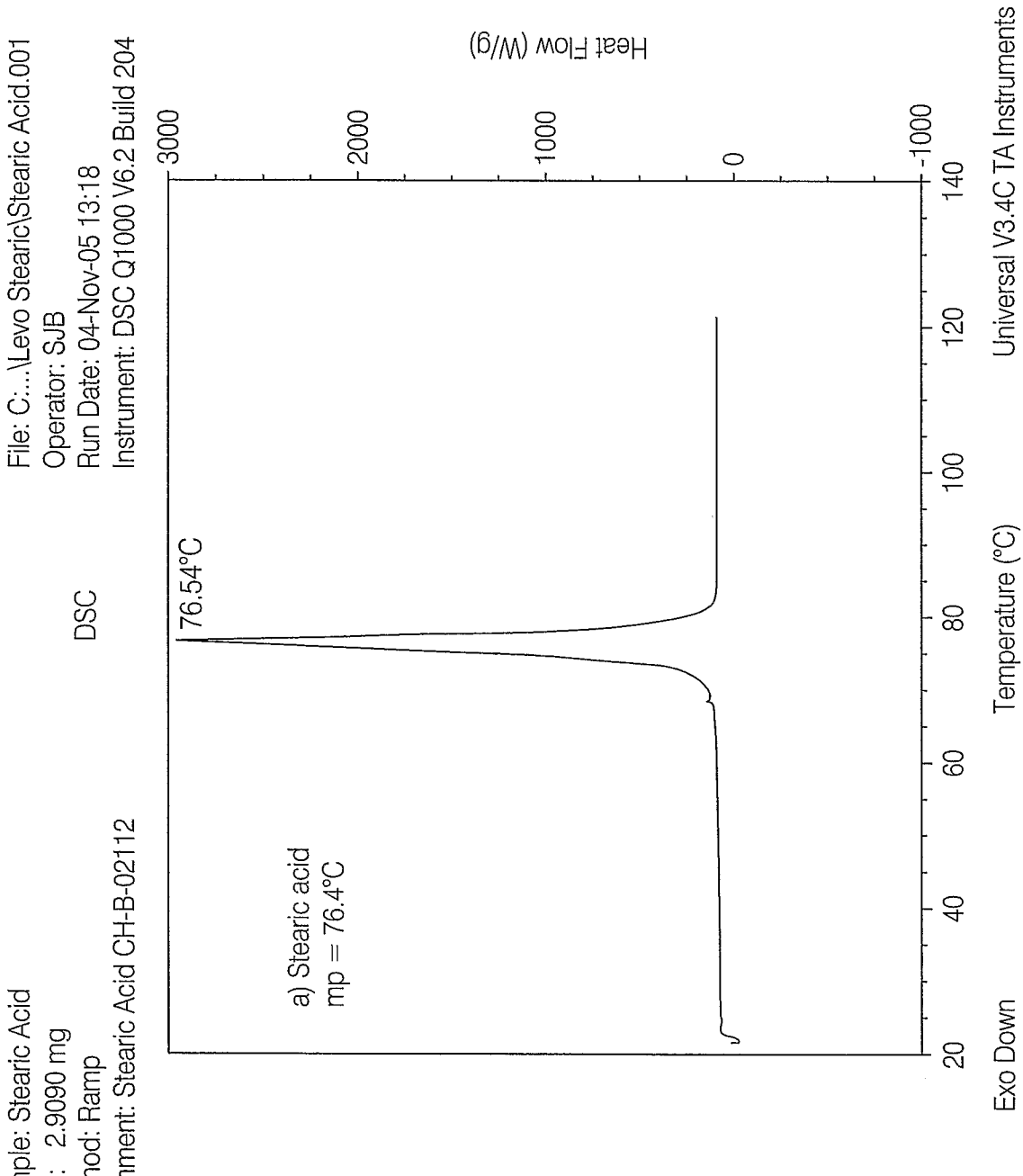

Sample: Stearic Acid
Size: 2.9090 mg
Method: Ramp
Comment: Stearic Acid CH-B-02112
DSC
File: C:...\Levo Stearic\Stearic Acid.001
Operator: SJB
Run Date: 04-Nov-05 13:18
Instrument: DSC Q1000 V6.2 Build 204
a) Stearic acid
mp = 76.4°C
76.54°C
Heat Flow (W/g)
3000
2000
1000
0
-1000
20
40
60
80
100
120
140
Temperature (°C)
Exo Down
Universal V3.4C TA Instruments

FIG. 26

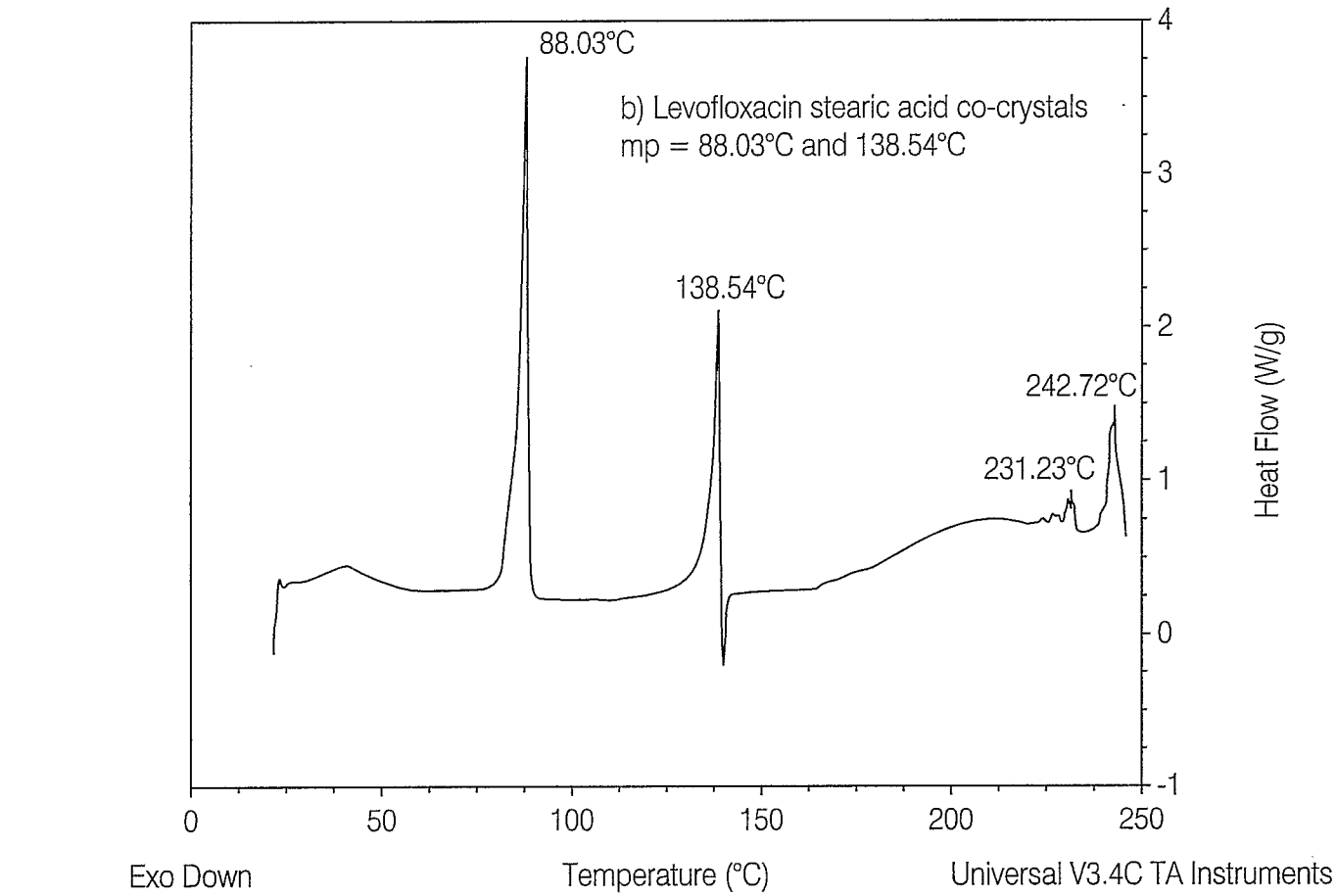


FIG. 26 continued

Sample: 049-001-01-114
Size: 2.7130 mg
Method: Ramp
Comment: Physical Mixture Stearic Acid + Levofloxacin Salt

DSC

File: ...\049-001-01-114@ 10 C per minute.001
Operator: SJB
Run Date: 15-Nov-05 12:46
Instrument: DSC Q1000 V6.2 Build 204

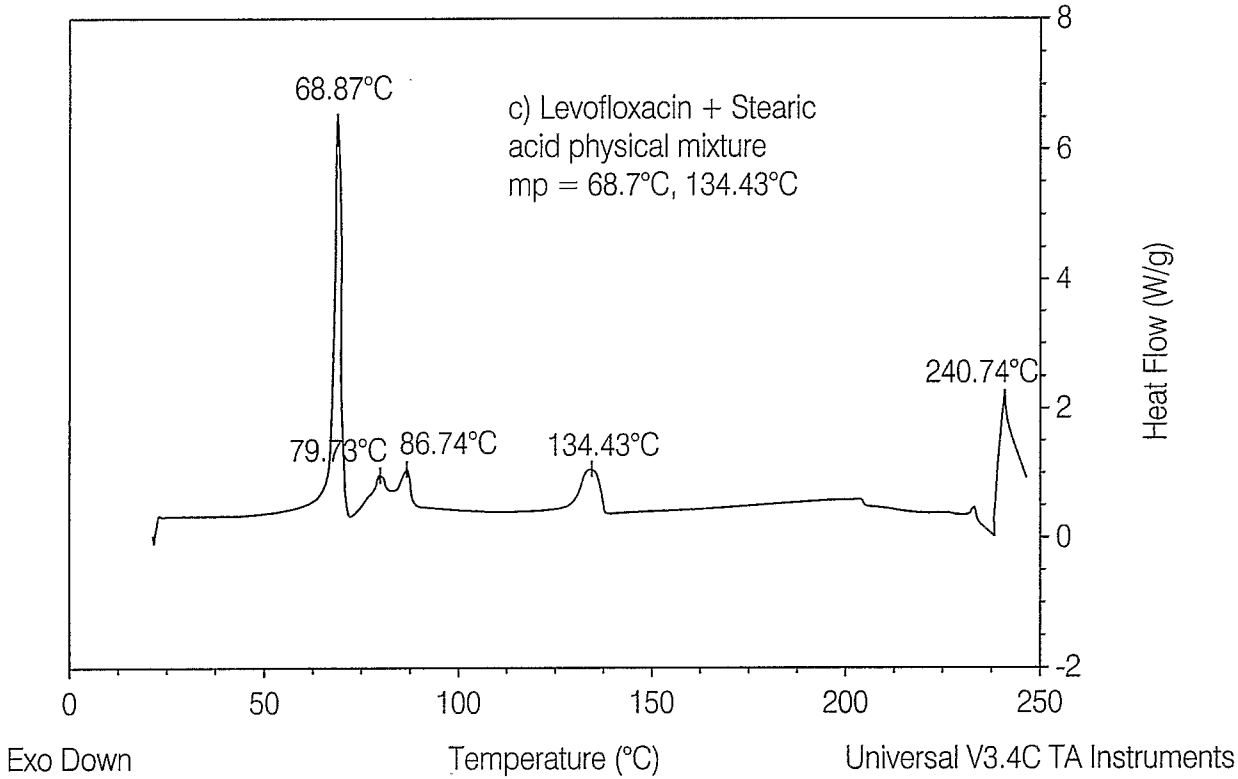


FIG. 26 continued

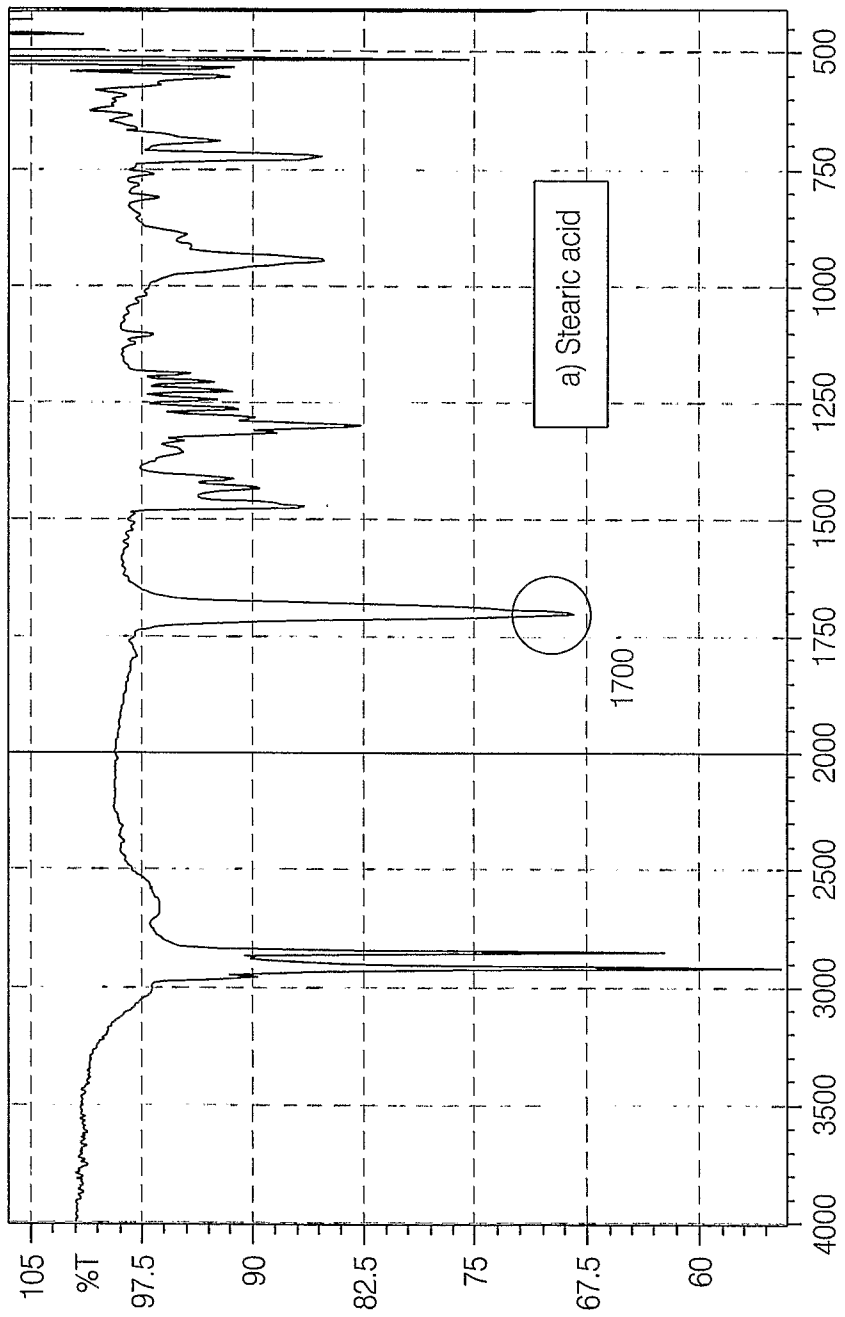

105
%T
97.5
90
82.5
75
67.5
60
4000
3500
3000
2500
2000
1750
1500
1250
1000
750
500
1/cm
a) Stearic acid
1700

FIG. 27

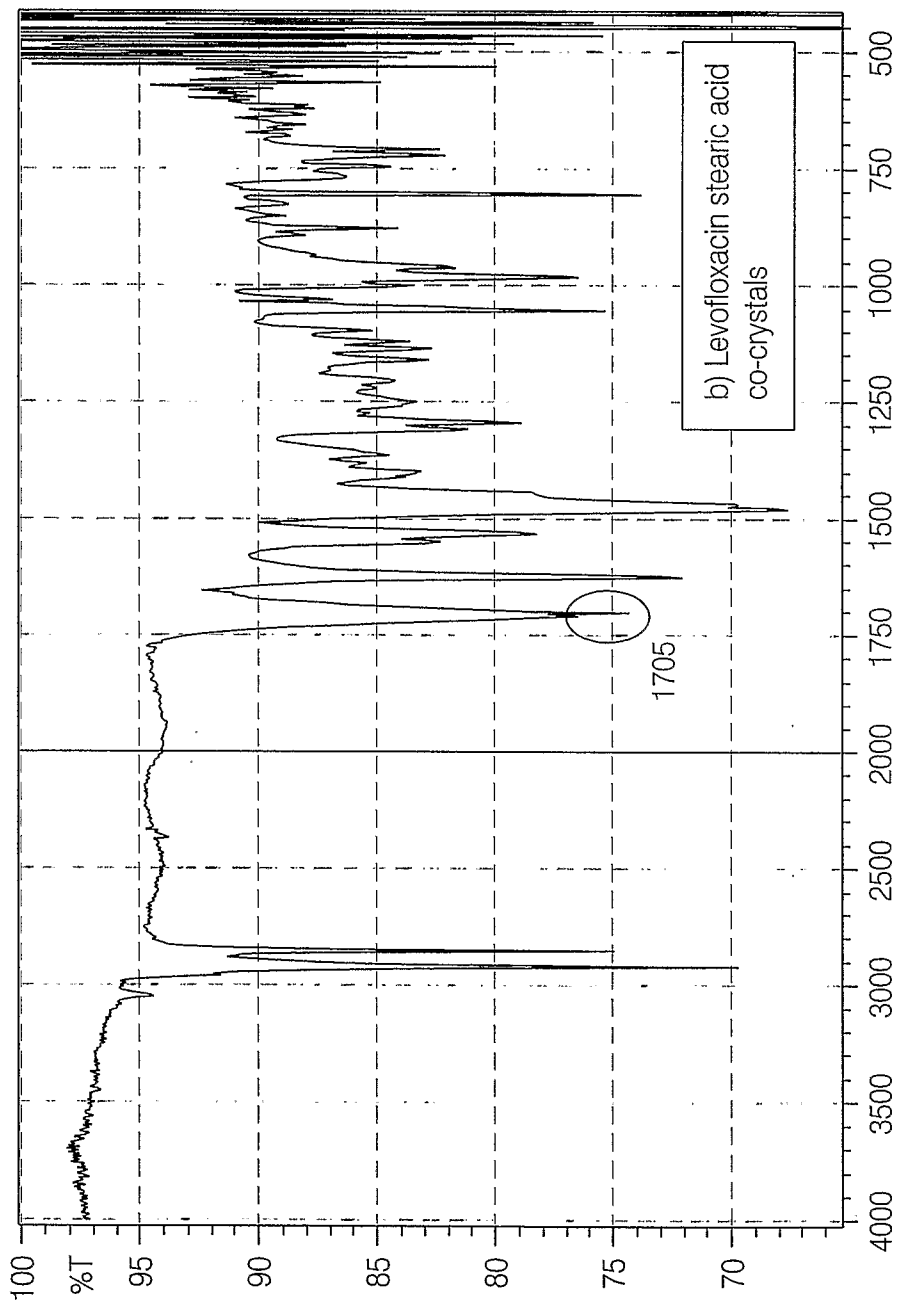


FIG. 27 continued

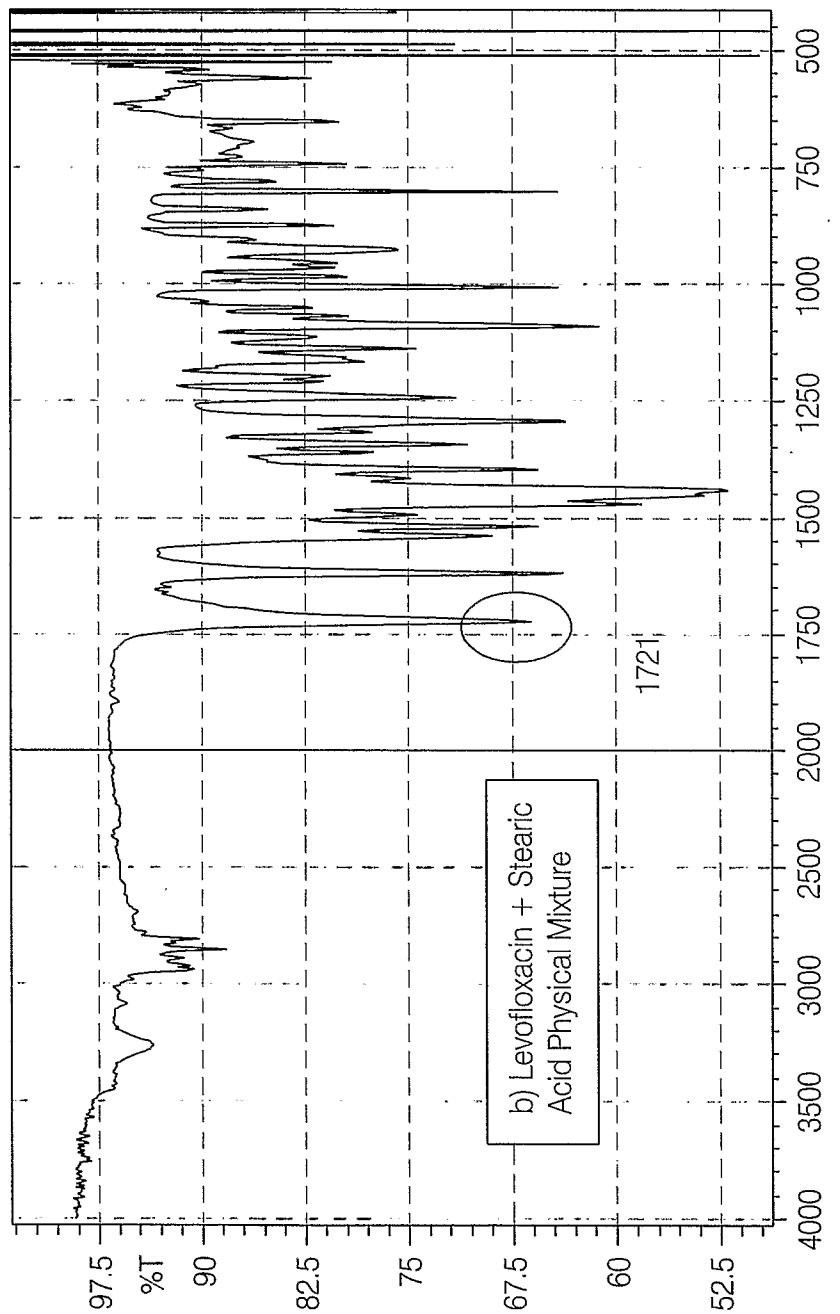


FIG. 27 continued

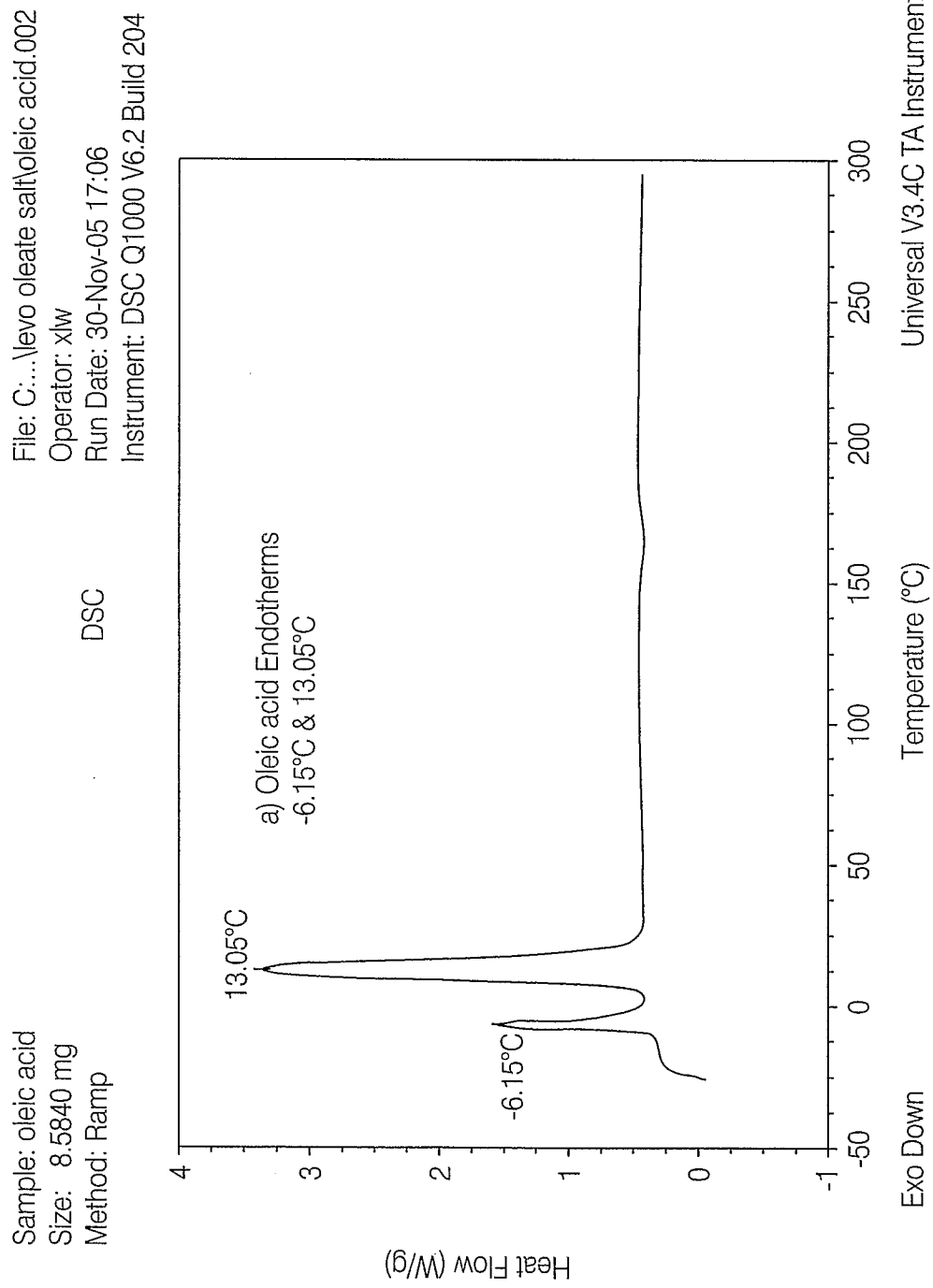

Sample: oleic acid
Size: 8.5840 mg
Method: Ramp
DSC
File: C:...\levo oleate salt\oleic acid.002
Operator: xlw
Run Date: 30-Nov-05 17:06
Instrument: DSC Q1000 V6.2 Build 204
a) Oleic acid Endotherms
-6.15°C & 13.05°C
13.05°C
-6.15°C
Heat Flow (W/g)
Exo Down
Temperature (°C)
Universal V3.4C TA Instruments

FIG. 28

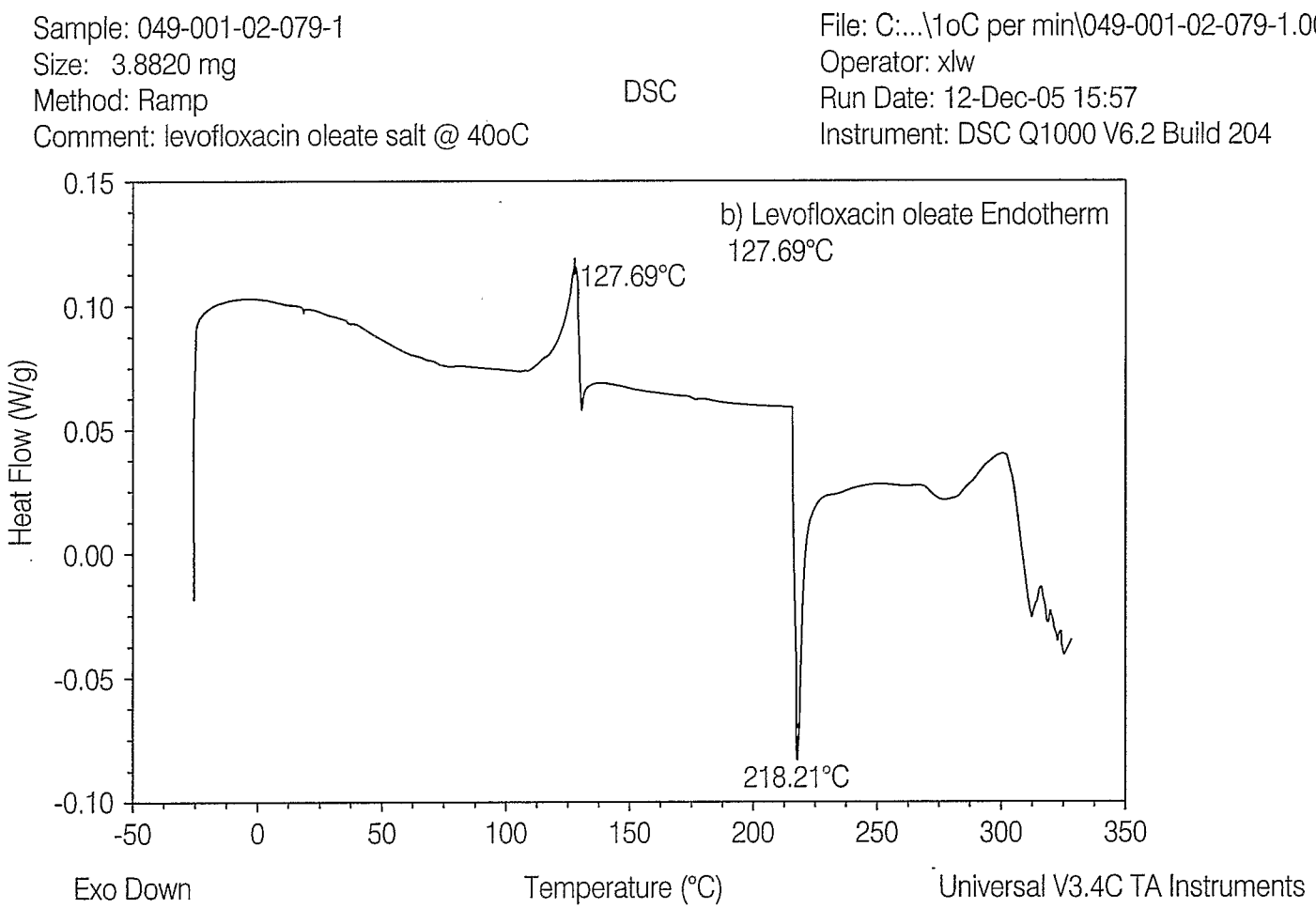


FIG. 28 continued

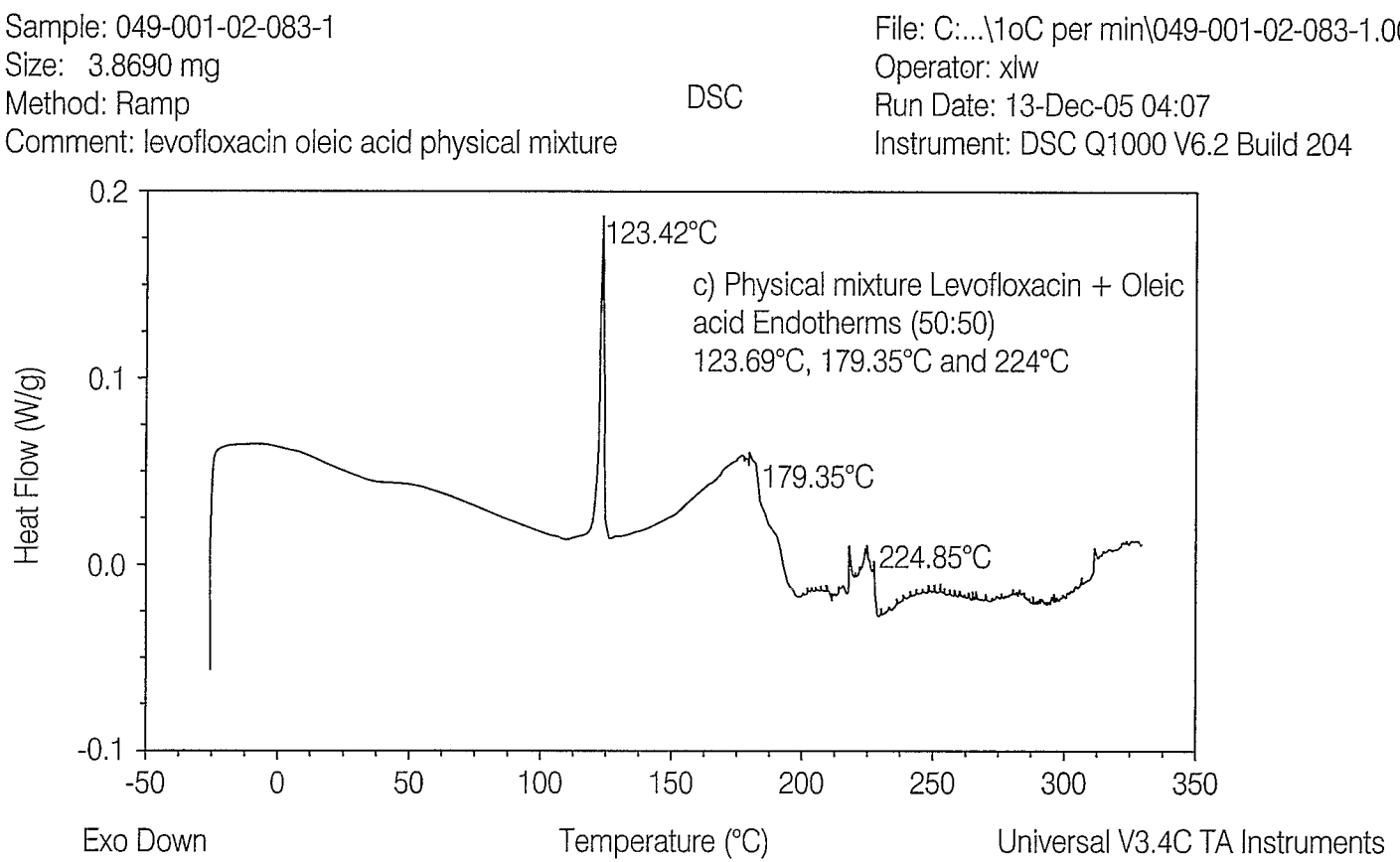


FIG. 28 continued

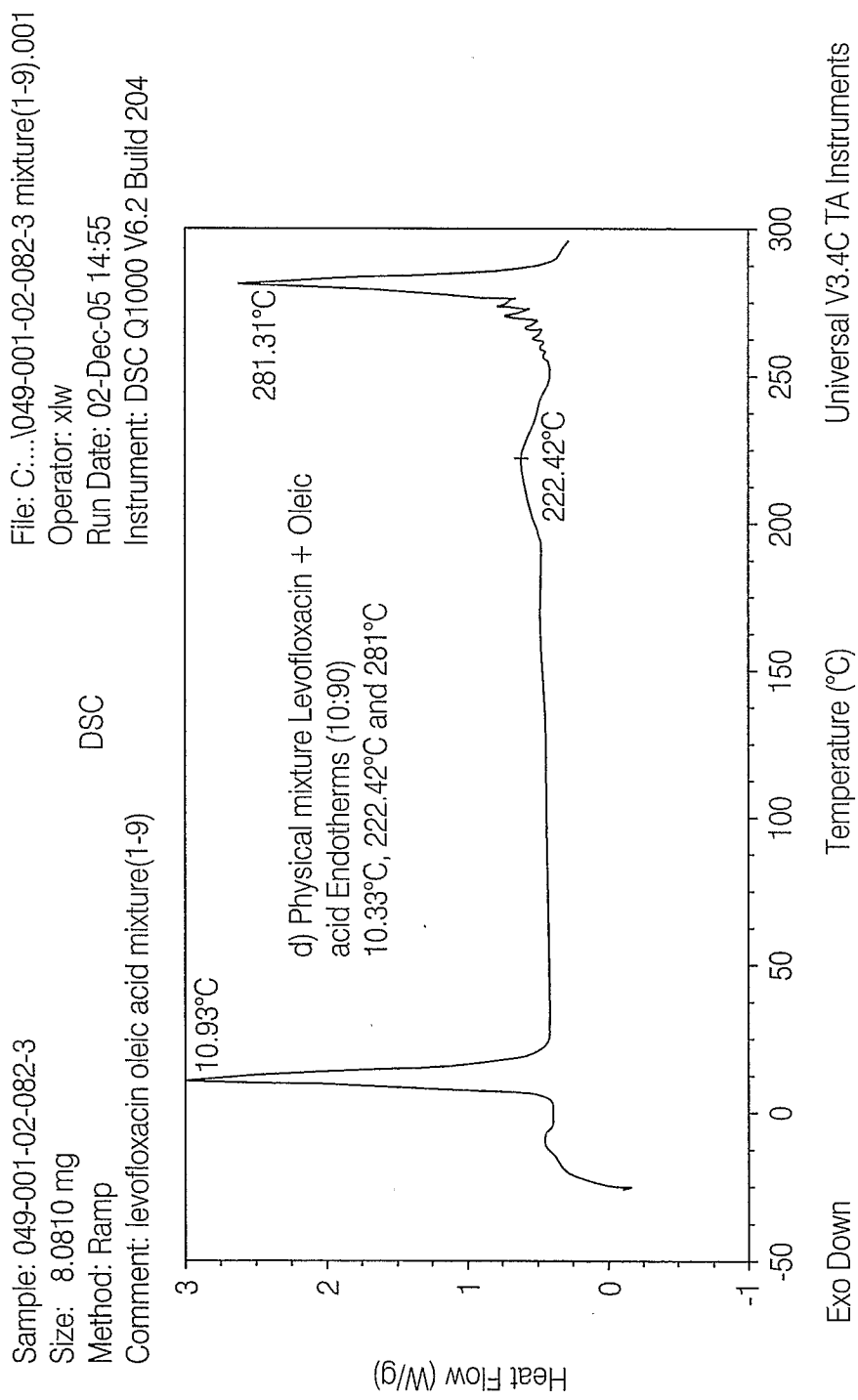


FIG. 28 continued

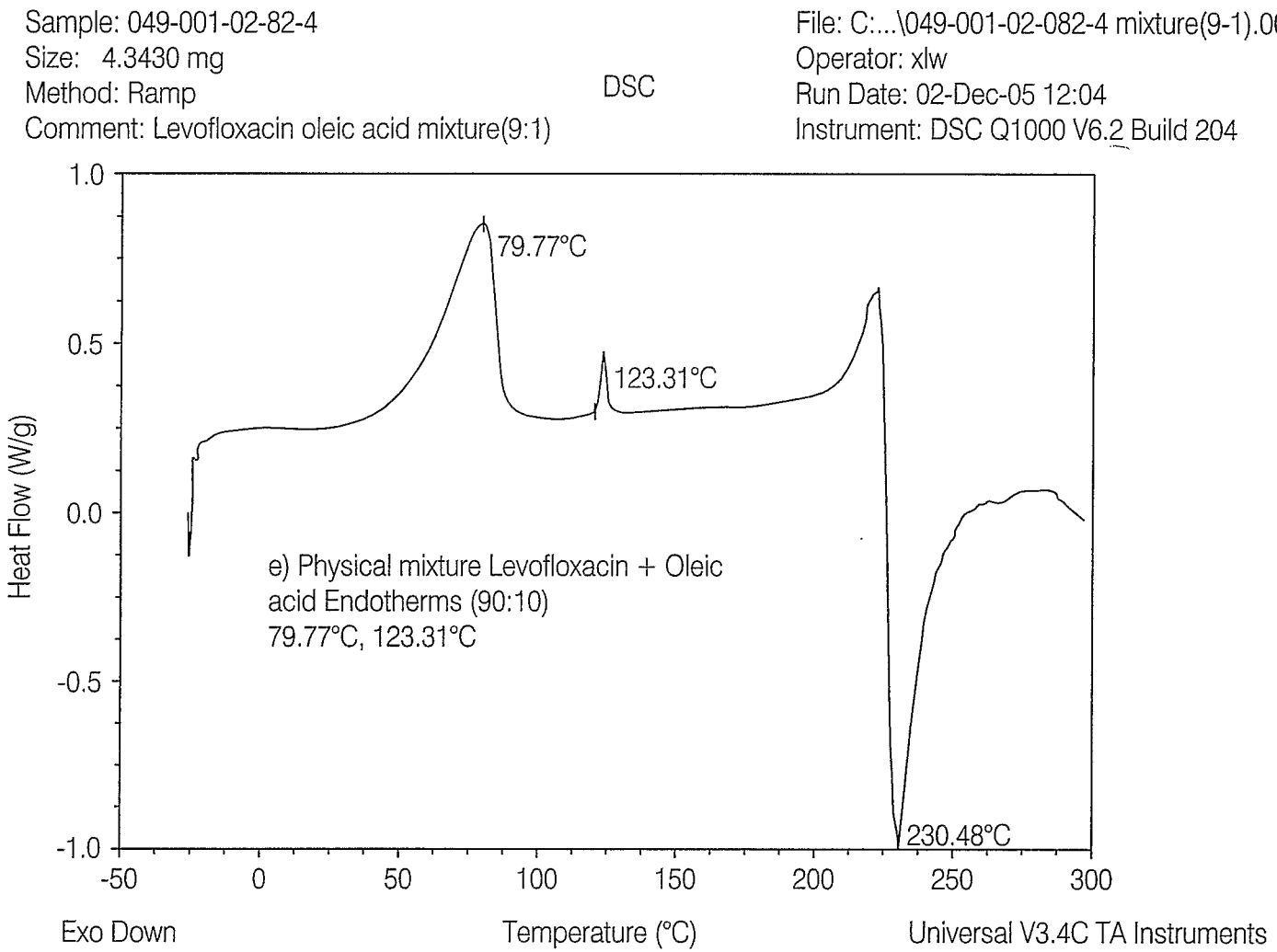


FIG. 28 continued

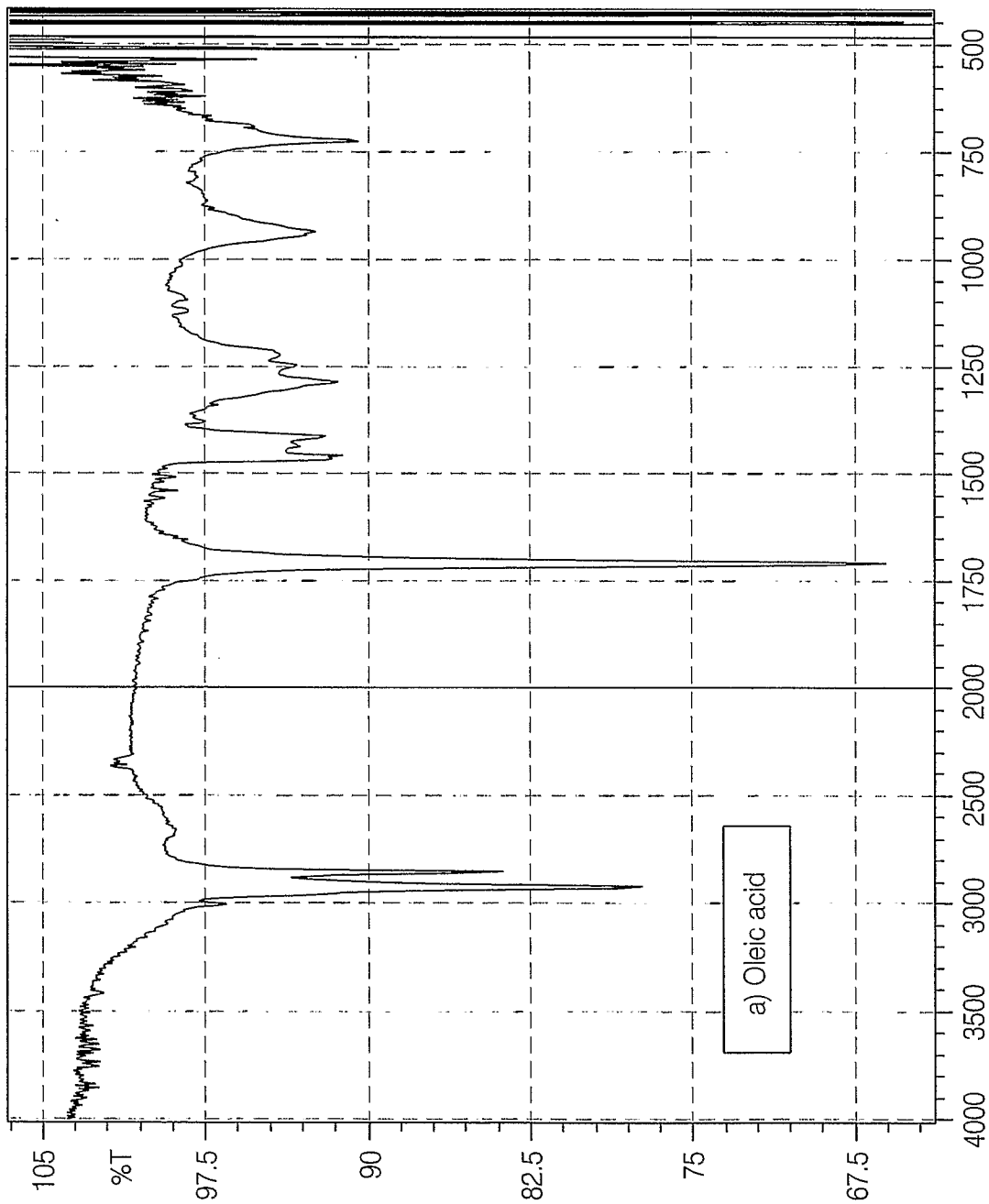

105
%T
97.5
90
82.5
75
67.5
4000
3500
3000
2500
2000
1750
1500
1250
1000
750
500
1/cm
a) Oleic acid

FIG. 29

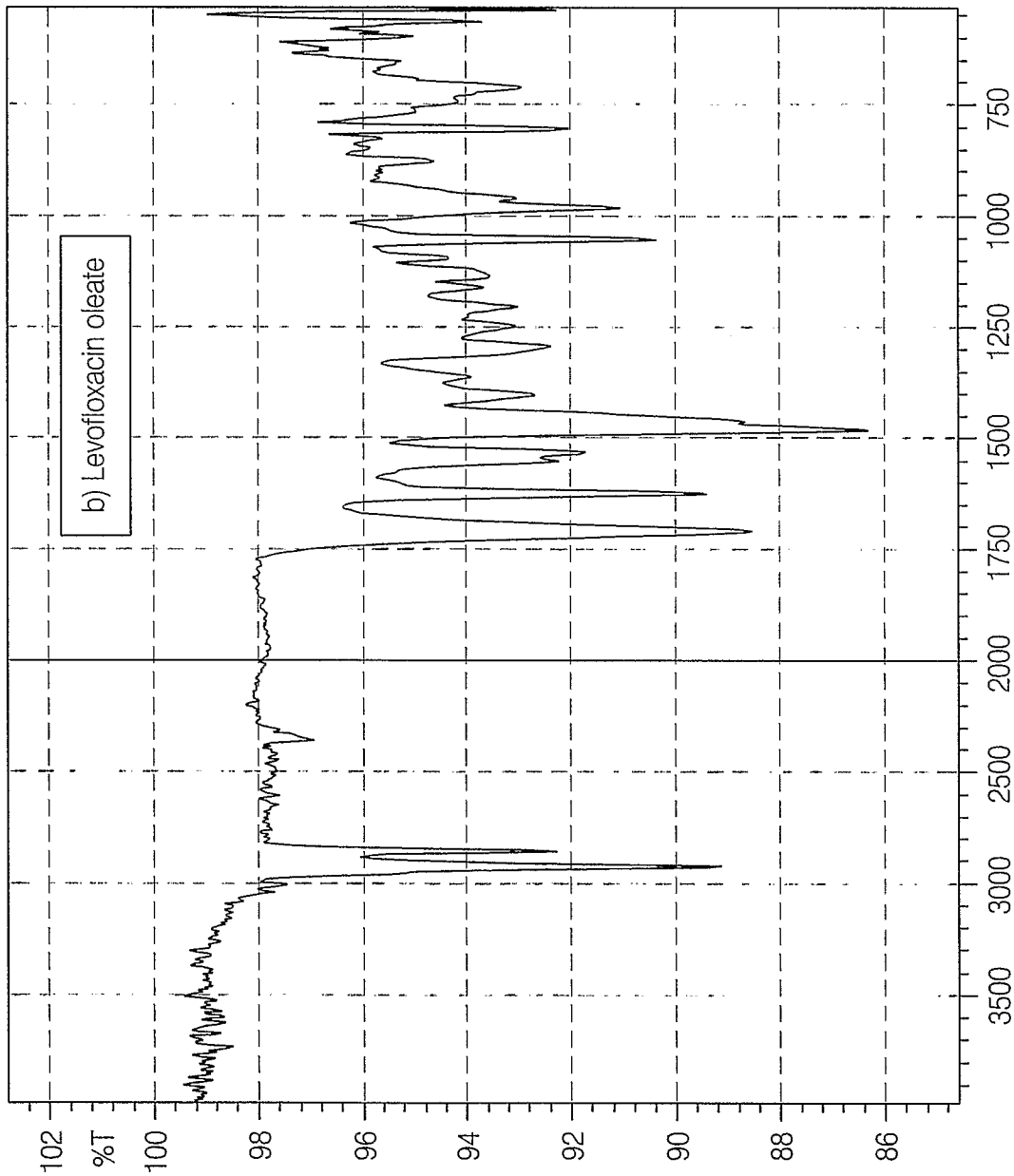


FIG. 29 continued

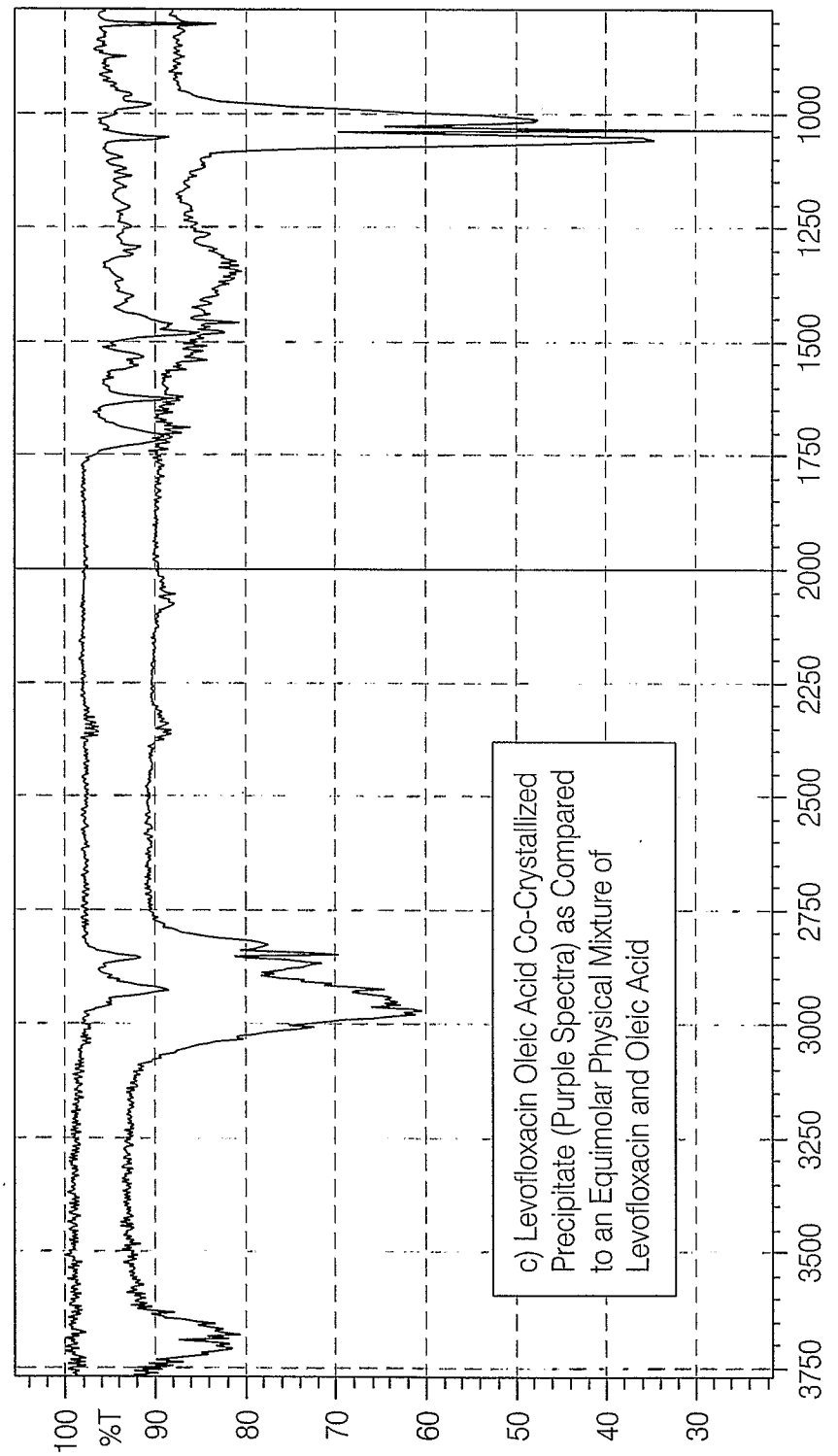


FIG. 29 continued

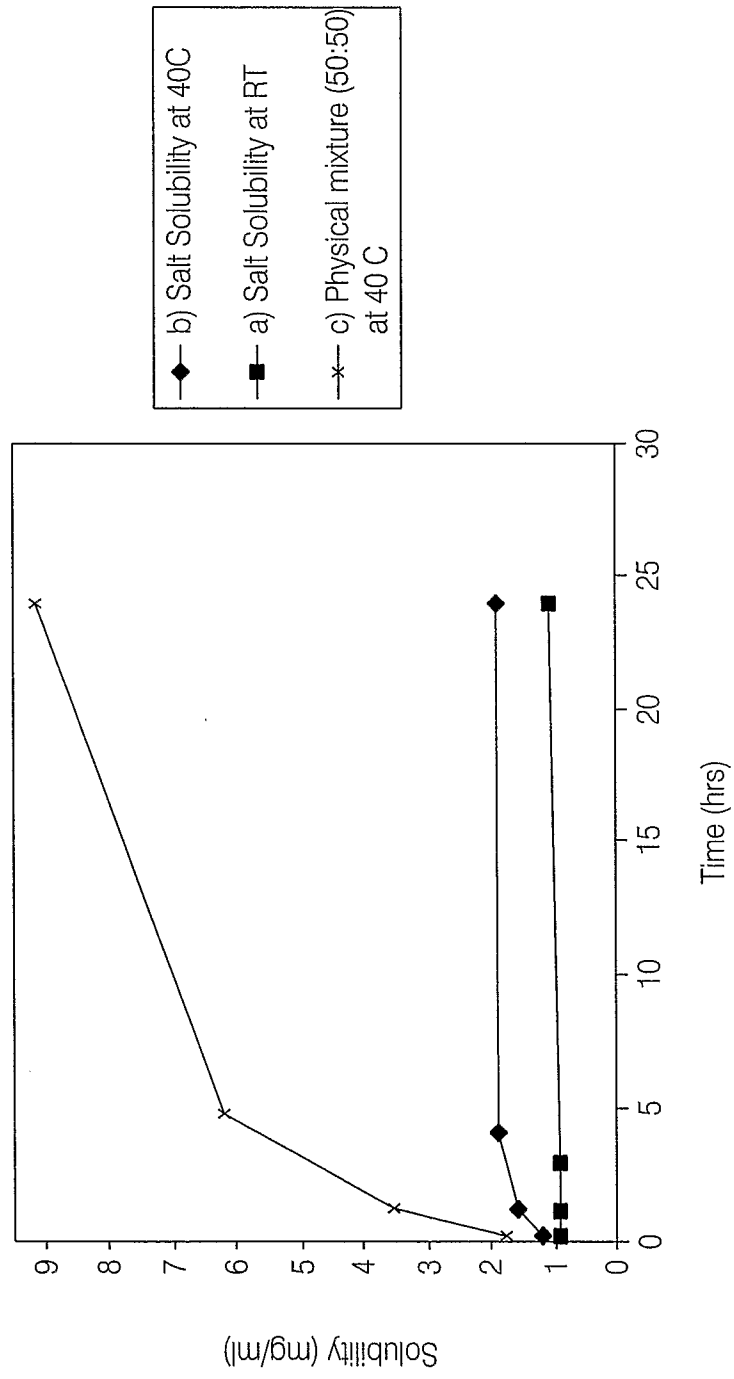

b) Salt Solubility at 40C
a) Salt Solubility at RT
c) Physical mixture (50:50) at 40 C
Solubility (mg/ml)
Time (hrs)
0
1
2
3
4
5
6
7
8
9
0
5
10
15
20
25
30

FIG. 30

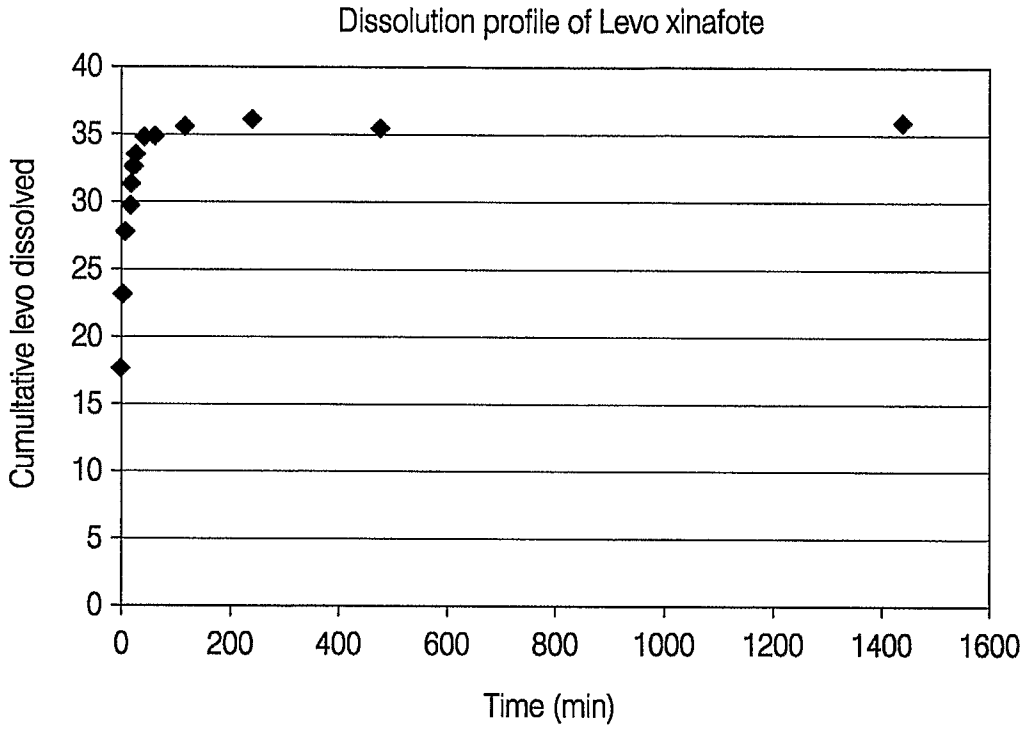

Dissolution profile of Levo xinafote
Cumultative levo dissolved
40
35
30
25
20
15
10
5
0
0
200
400
600
800
1000
1200
1400
1600
Time (min)

FIG. 31

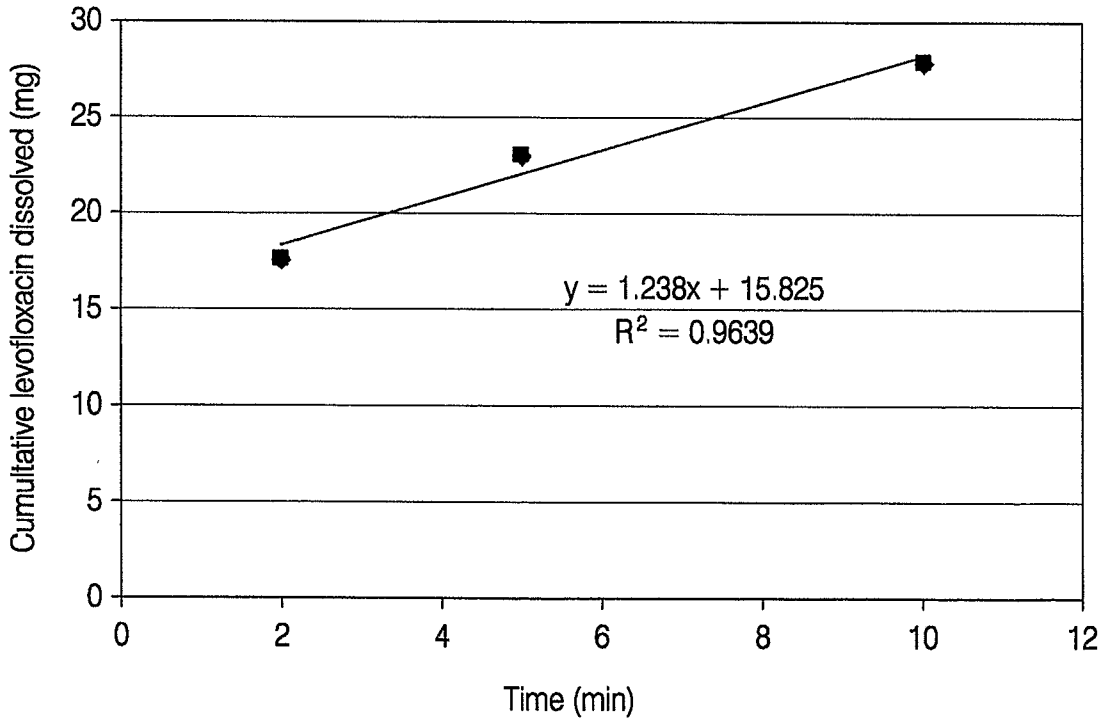

Cumultative levofloxacin dissolved (mg)
30
25
20
15
10
5
0
y = 1.238x + 15.825
R2 = 0.9639
0
2
4
6
8
10
12
Time (min)

FIG. 32

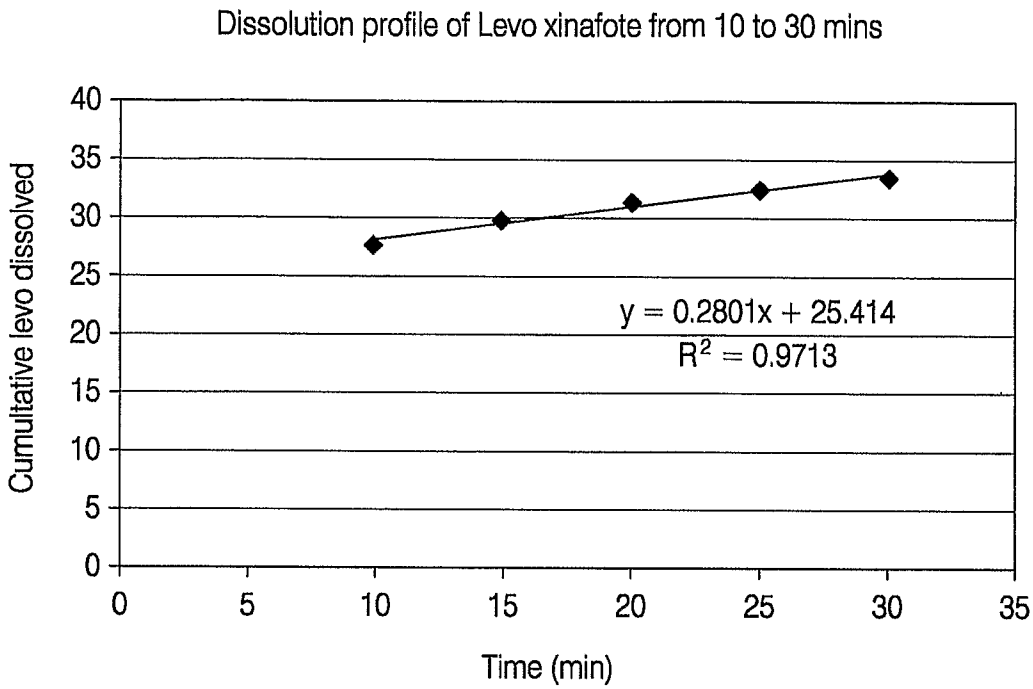

Dissolution profile of Levo xinafote from 10 to 30 mins
Cumultative levo dissolved
40
35
30
25
20
15
10
5
0
0
5
10
15
20
25
30
35
Time (min)
y = 0.2801x + 25.414
R2 = 0.9713

FIG. 33

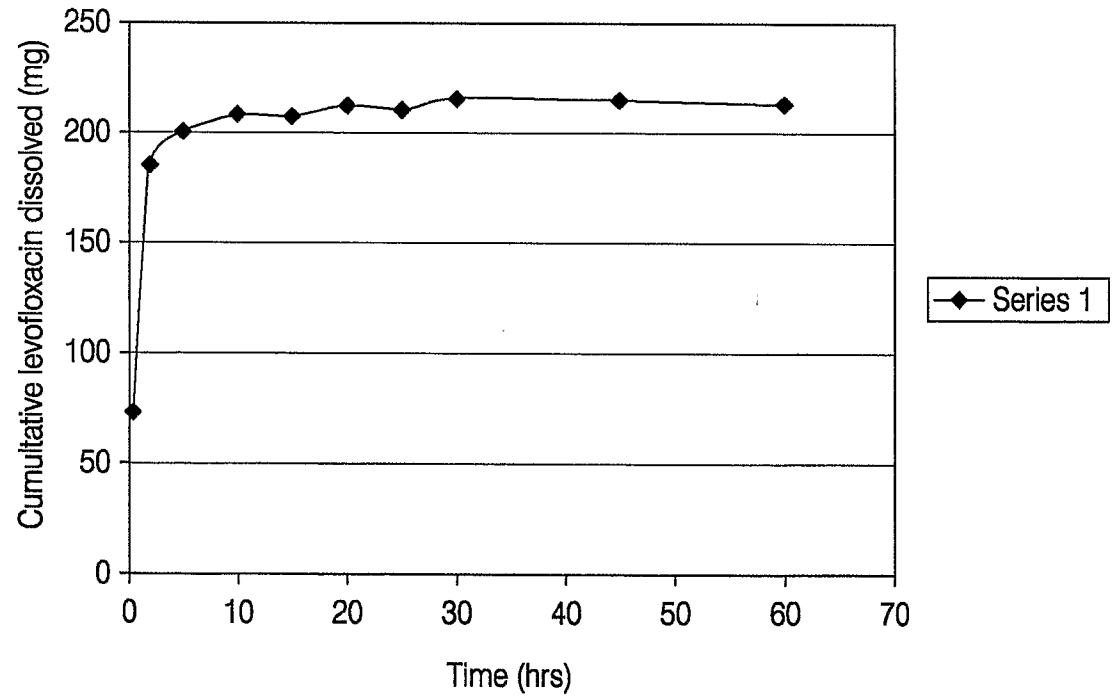

Cumultative levofloxacin dissolved (mg)
250
200
150
100
50
0
0
10
20
30
40
50
60
70
Time (hrs)
Series 1

FIG. 34

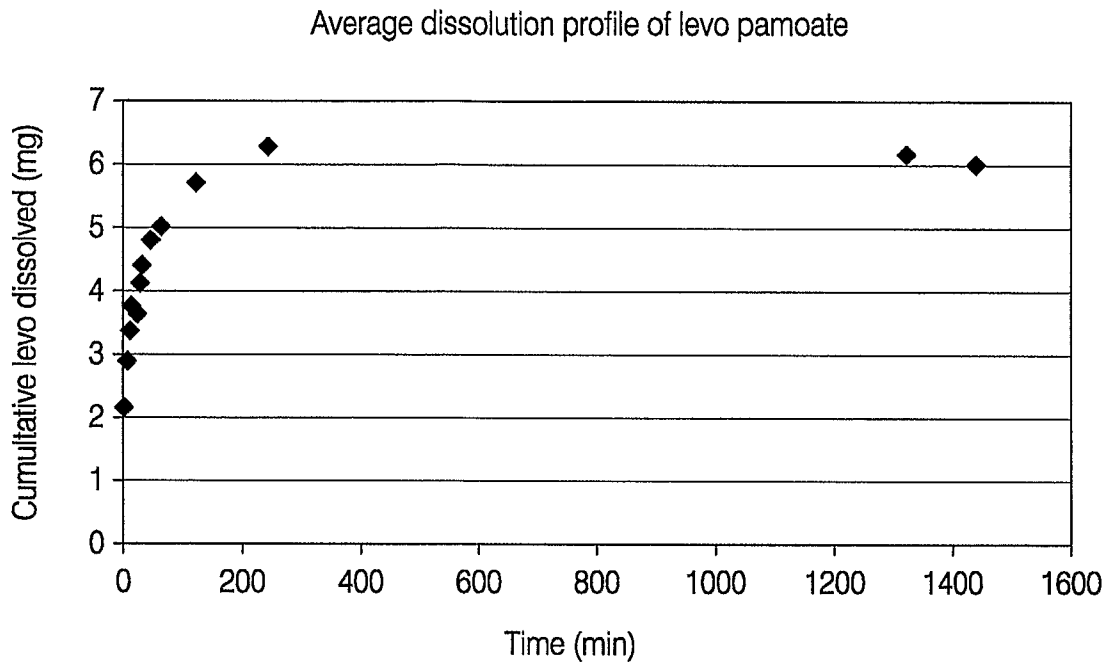

Average dissolution profile of levo pamoate
Cumulative levo dissolved (mg)
0
1
2
3
4
5
6
7
0
200
400
600
800
1000
1200
1400
1600
Time (min)

FIG. 35

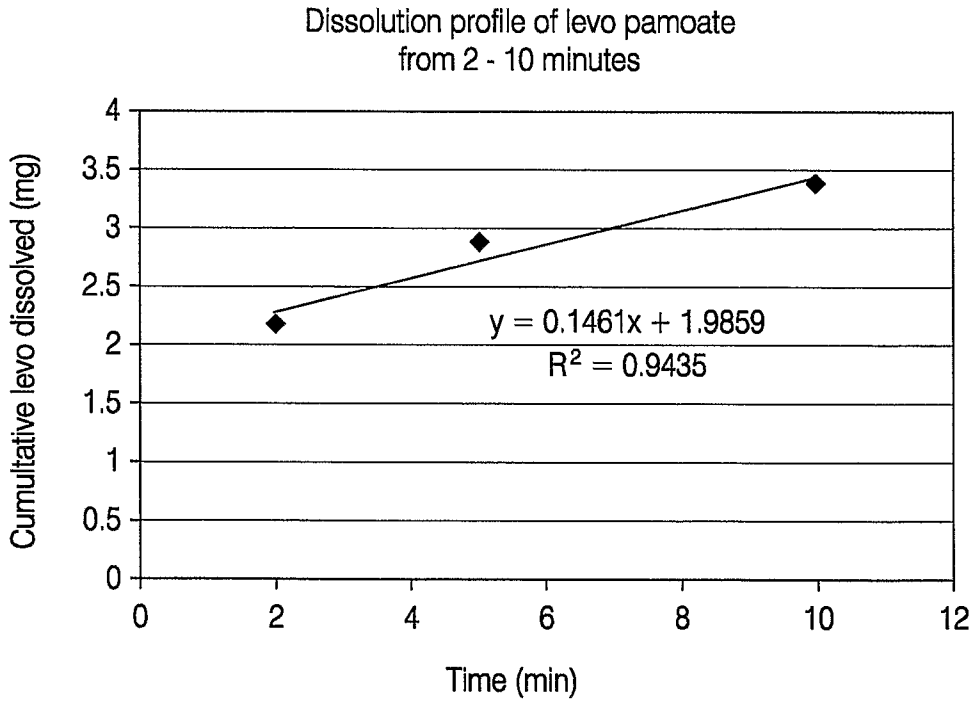

Dissolution profile of levo pamoate
from 2 - 10 minutes
Cumulative levo dissolved (mg)
0
0.5
1
1.5
2
2.5
3
3.5
4
0
2
4
6
8
10
12
y = 0.1461x + 1.9859
R2 = 0.9435
Time (min)

FIG. 36

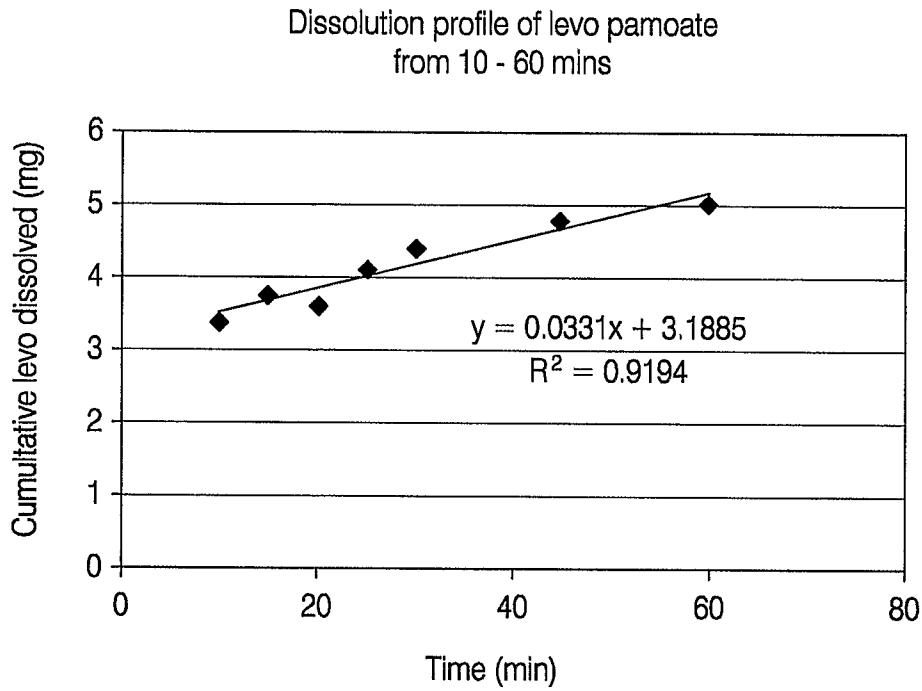

Dissolution profile of levo pamoate
from 10 - 60 mins
Cumultative levo dissolved (mg)
6
5
4
3
2
1
0
0
20
40
60
80
Time (min)
y = 0.0331x + 3.1885
R2 = 0.9194

FIG. 37

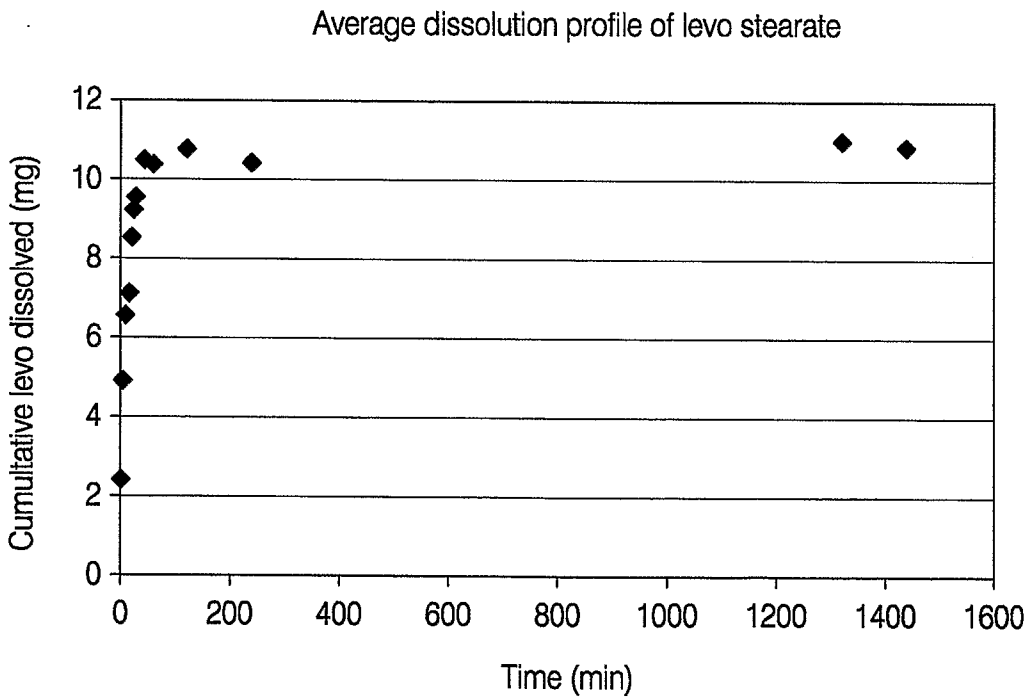

Average dissolution profile of levo stearate
Cumultative levo dissolved (mg)
12
10
8
6
4
2
0
0
200
400
600
800
1000
1200
1400
1600
Time (min)

FIG. 38

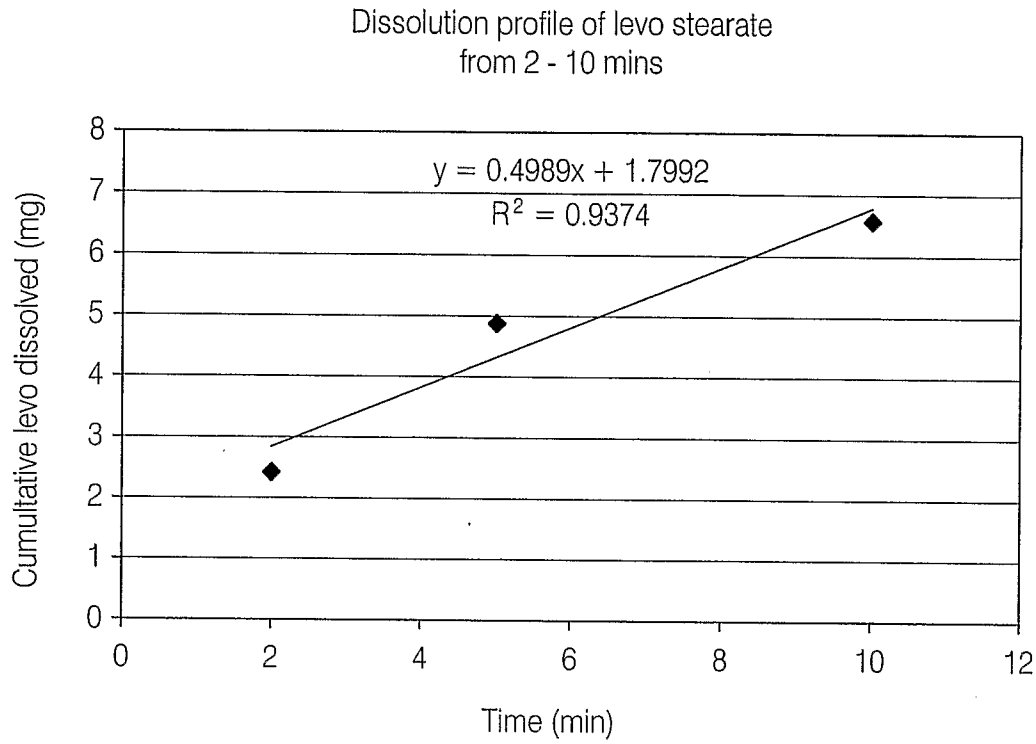

Dissolution profile of levo stearate
from 2 - 10 mins
y = 0.4989x + 1.7992
R2 = 0.9374
Cumultative levo dissolved (mg)
Time (min)
0
1
2
3
4
5
6
7
8
0
2
4
6
8
10
12

FIG. 39

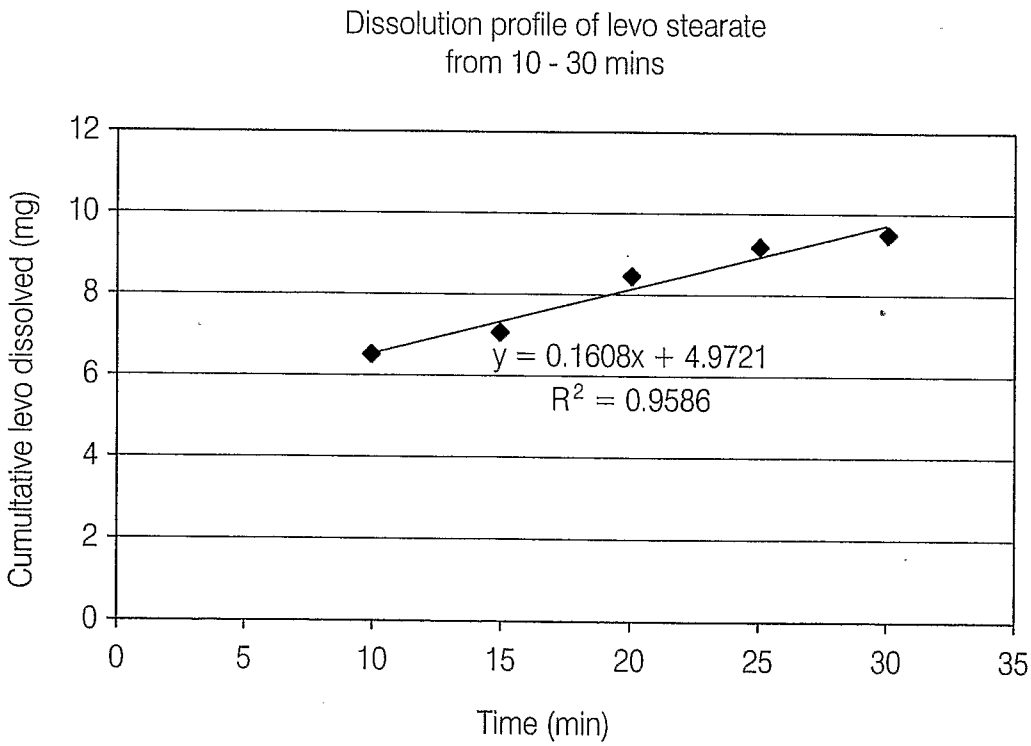

Dissolution profile of levo stearate
from 10 - 30 mins
y = 0.1608x + 4.9721
R2 = 0.9586
Cumultative levo dissolved (mg)
Time (min)
0
2
4
6
8
10
12
0
5
10
15
20
25
30
35

FIG. 40

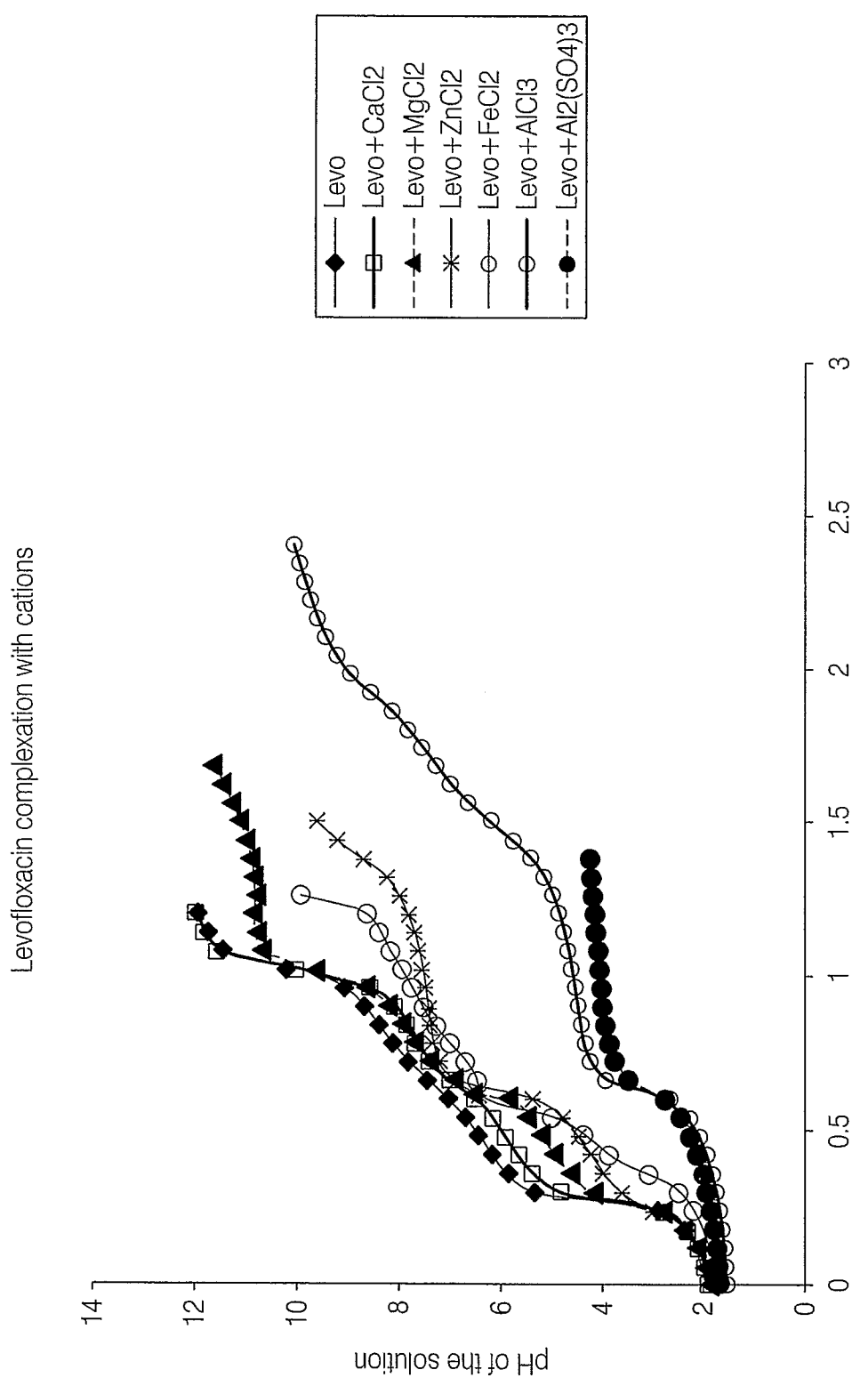

Levofloxacin complexation with cations
pH of the solution
mmoles of NaOH added
Levo
Levo+CaCl2
Levo+MgCl2
Levo+ZnCl2
Levo+FeCl2
Levo+AlCl3
Levo+Al2(SO4)3
0
2
4
6
8
10
12
14
0
0.5
1
1.5
2
2.5
3

FIG. 41

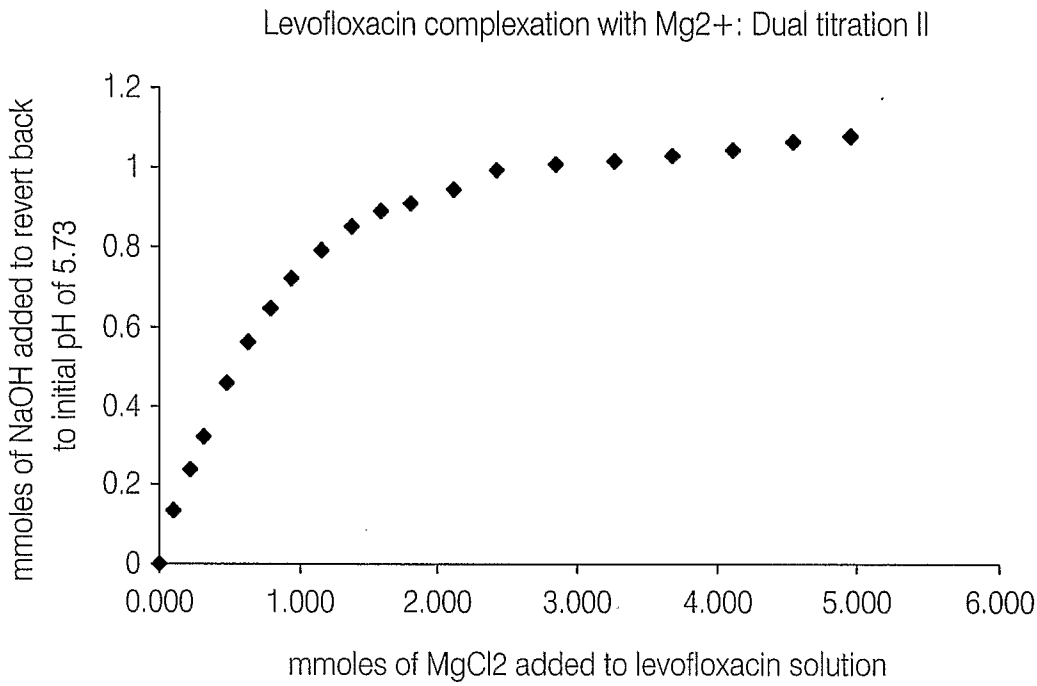

Levofloxacin complexation with Mg2+: Dual titration II
mmoles of NaOH added to revert back to initial pH of 5.73
1.2
1
0.8
0.6
0.4
0.2
0
0.000
1.000
2.000
3.000
4.000
5.000
6.000
mmoles of MgCl2 added to levofloxacin solution

FIG. 42

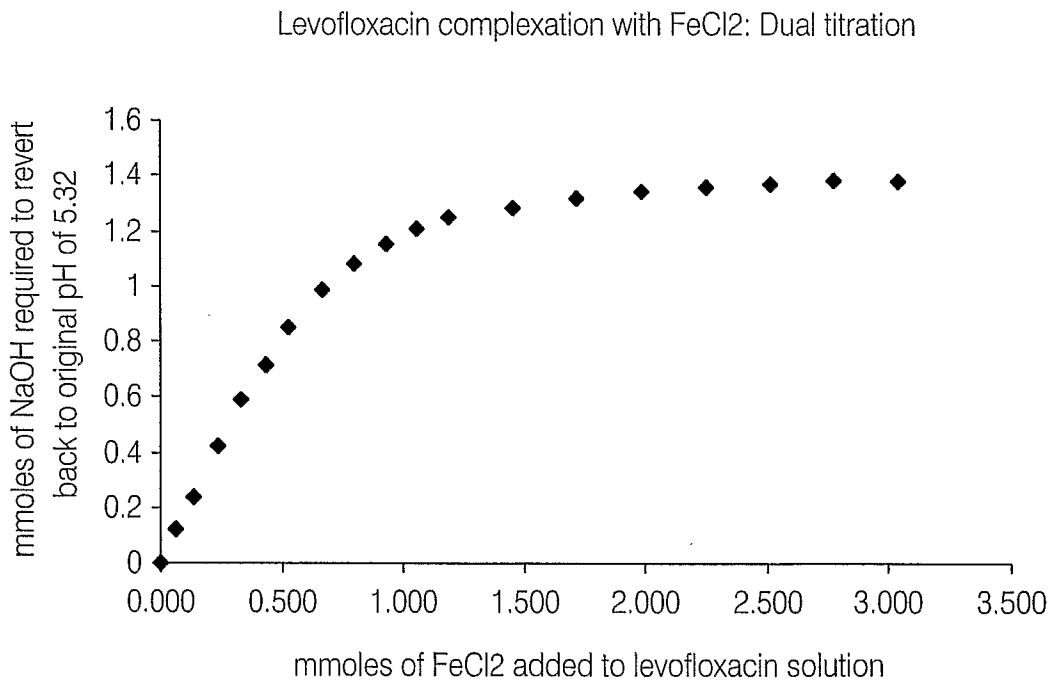

Levofloxacin complexation with FeCl2: Dual titration
mmoles of NaOH required to revert back to original pH of 5.32
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.000
0.500
1.000
1.500
2.000
2.500
3.000
3.500
mmoles of FeCl2 added to levofloxacin solution

FIG. 43

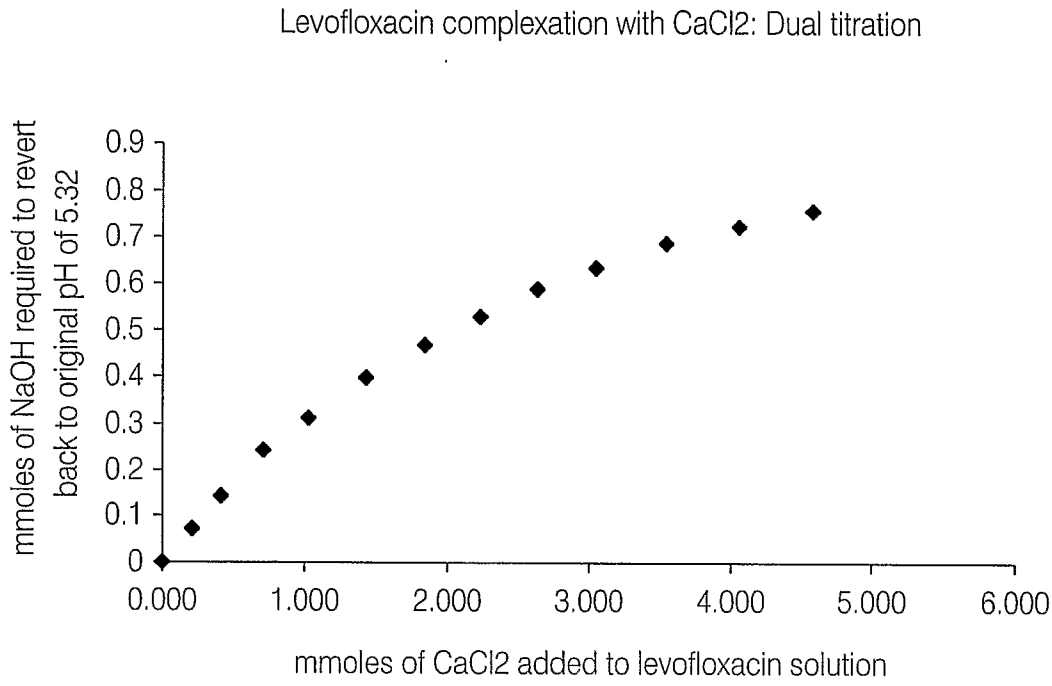

Levofloxacin complexation with CaCl2: Dual titration
mmoles of NaOH required to revert back to original pH of 5.32
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.000
1.000
2.000
3.000
4.000
5.000
6.000
mmoles of CaCl2 added to levofloxacin solution

FIG. 44

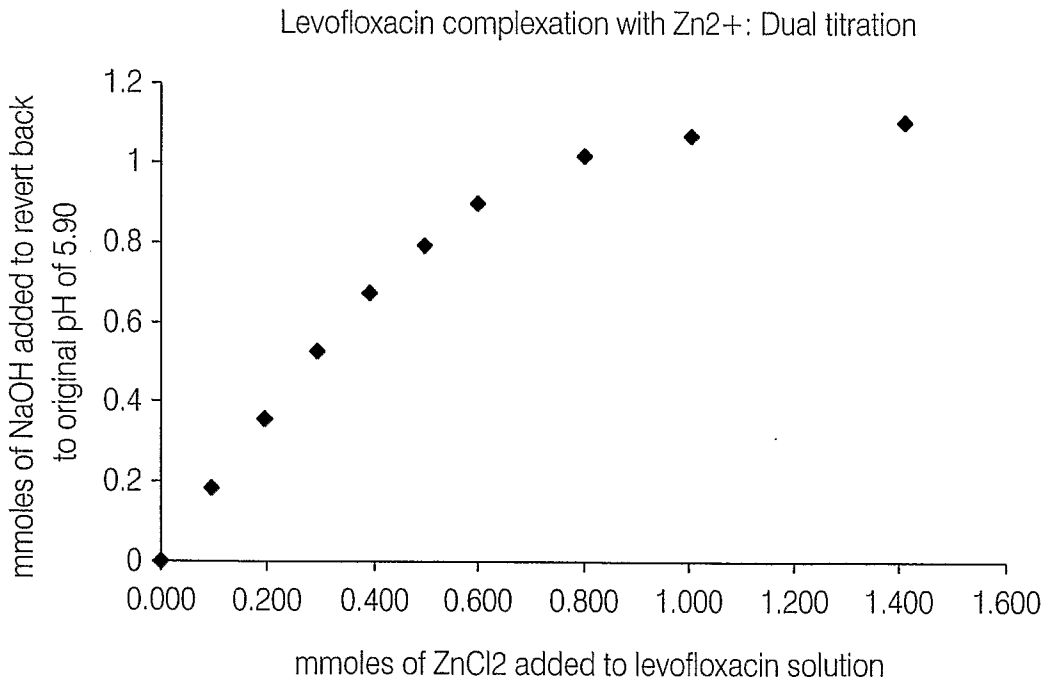
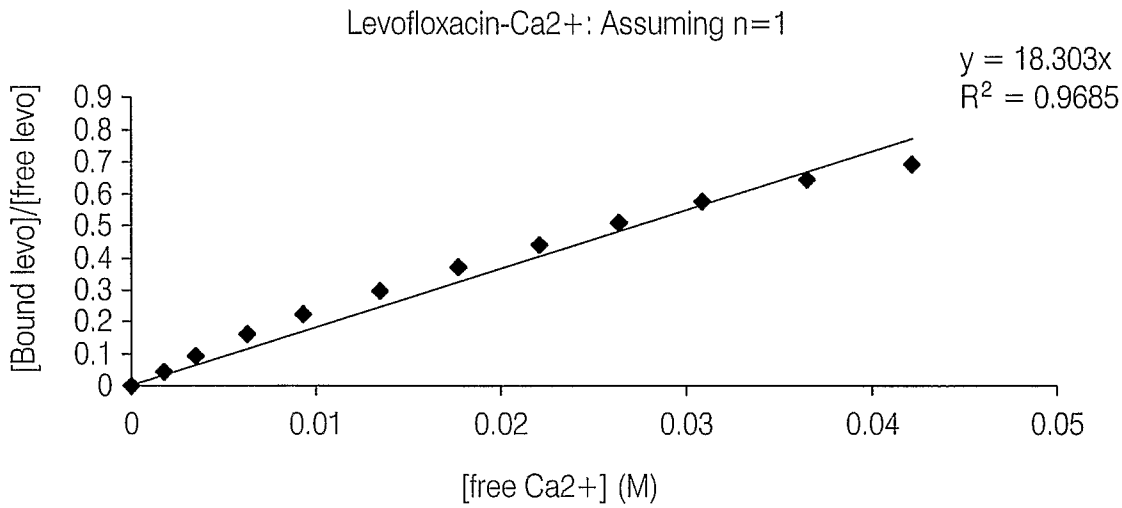

Levofloxacin complexation with Zn2+: Dual titration
mmoles of NaOH added to revert back to original pH of 5.90
1.2
1
0.8
0.6
0.4
0.2
0
0.000
0.200
0.400
0.600
0.800
1.000
1.200
1.400
1.600
mmoles of ZnCl2 added to levofloxacin solution

FIG. 45


Levofloxacin-Ca2+: Assuming n=1
y = 18.303x
R2 = 0.9685
[Bound levo]/[free levo]
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.01
0.02
0.03
0.04
0.05
[free Ca2+] (M)

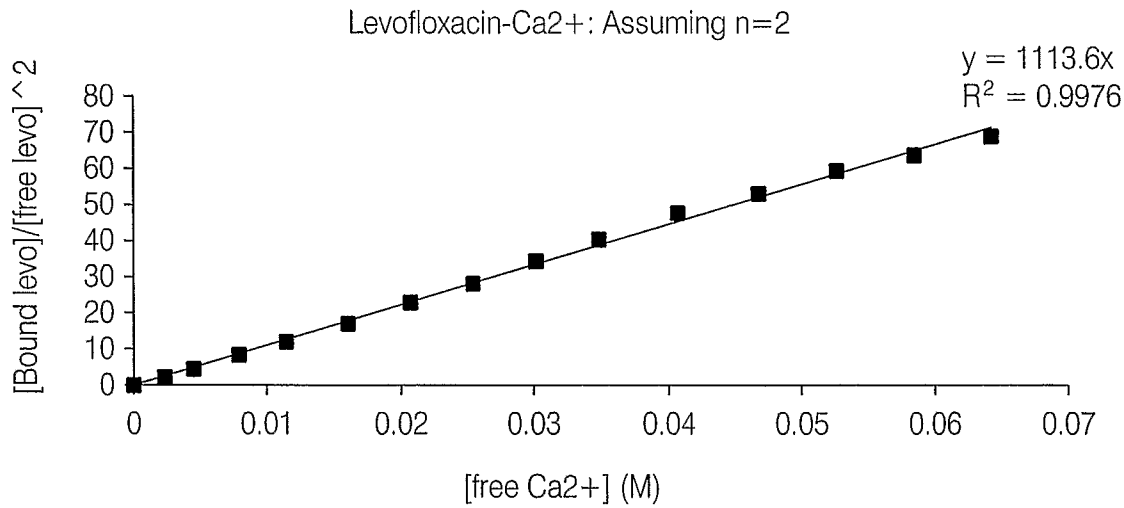
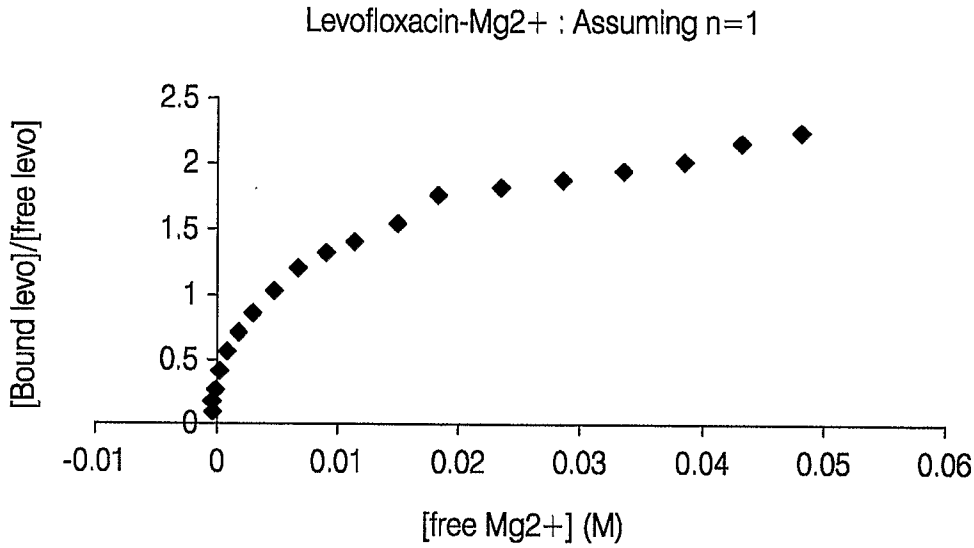

Levofloxacin-Ca2+: Assuming n=2
y = 1113.6x
R2 = 0.9976
[Bound levo]/[free levo] ^ 2
80
70
60
50
40
30
20
10
0
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
[free Ca2+] (M)

FIG. 46


Levofloxacin-Mg2+ : Assuming n=1
[Bound levo]/[free levo]
2.5
2
1.5
1
0.5
0
-0.01
0
0.01
0.02
0.03
0.04
0.05
0.06
[free Mg2+] (M)

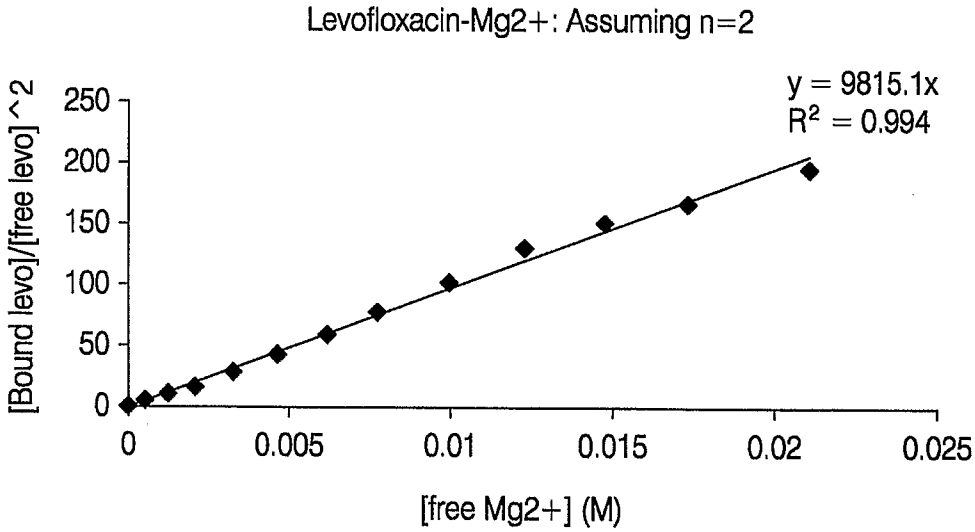
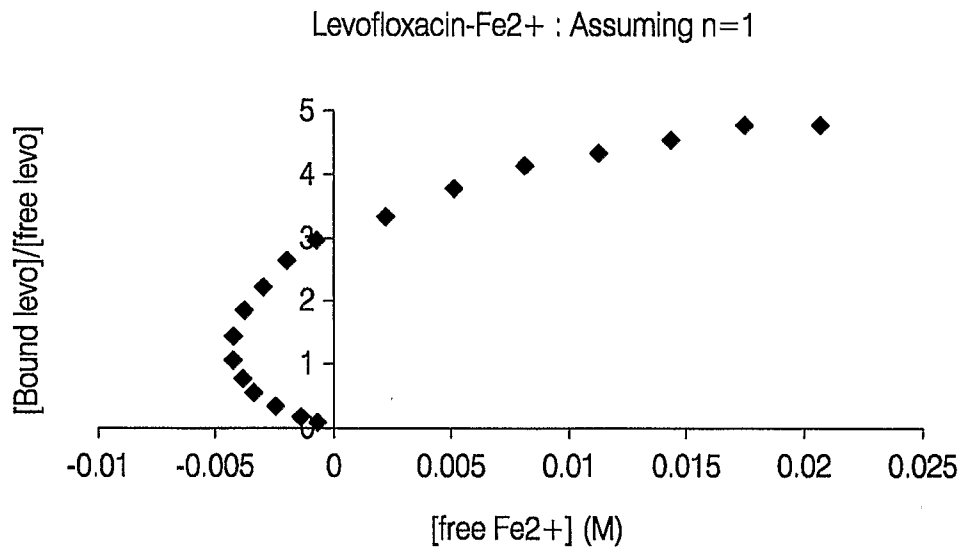

Levofloxacin-Mg2+: Assuming n=2
y = 9815.1x
R2 = 0.994
[Bound levo]/[free levo] ^2
250
200
150
100
50
0
0
0.005
0.01
0.015
0.02
0.025
[free Mg2+] (M)

FIG. 47


Levofloxacin-Fe2+ : Assuming n=1
[Bound levo]/[free levo]
5
4
3
2
1
0
-0.01
-0.005
0
0.005
0.01
0.015
0.02
0.025
[free Fe2+] (M)

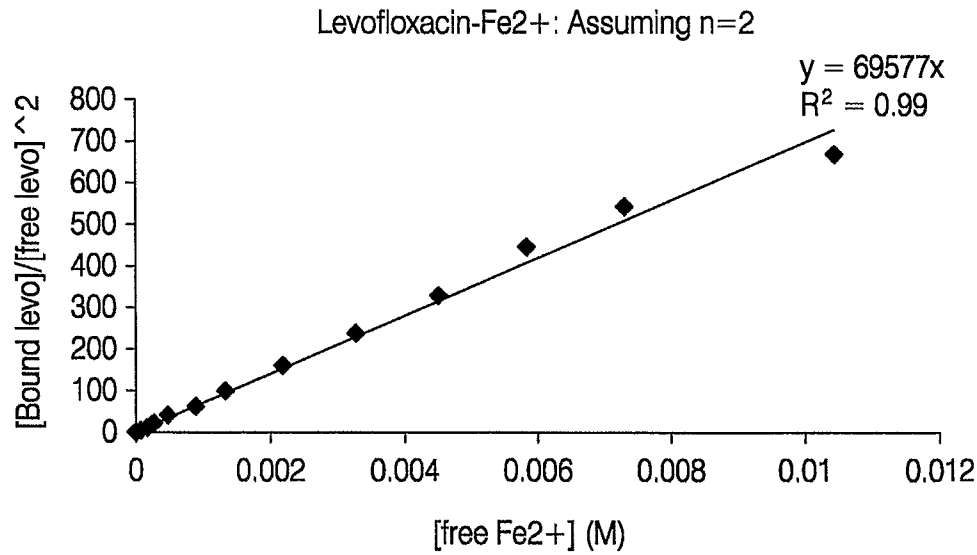
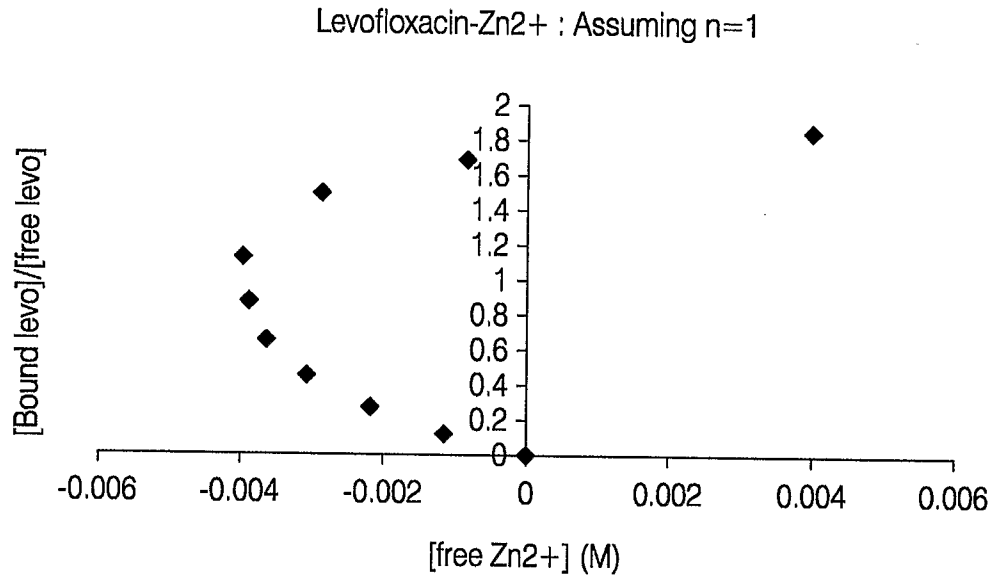

Levofloxacin-Fe2+: Assuming n=2
y = 69577x
R2 = 0.99
[Bound levo]/[free levo] ^2
800
700
600
500
400
300
200
100
0
0
0.002
0.004
0.006
0.008
0.01
0.012
[free Fe2+] (M)

FIG. 48


Levofloxacin-Zn2+ : Assuming n=1
[Bound levo]/[free levo]
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.006
-0.004
-0.002
0
0.002
0.004
0.006
[free Zn2+] (M)

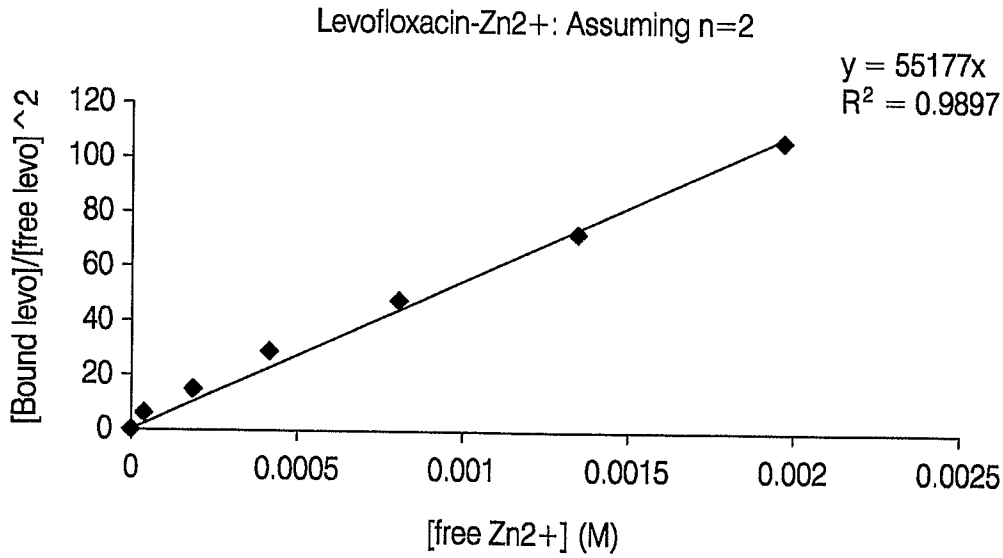

Levofloxacin-Zn2+: Assuming n=2
y = 55177x
R2 = 0.9897
[Bound levo]/[free levo] ^2
120
100
80
60
40
20
0
0
0.0005
0.001
0.0015
0.002
0.0025
[free Zn2+] (M)

FIG. 49

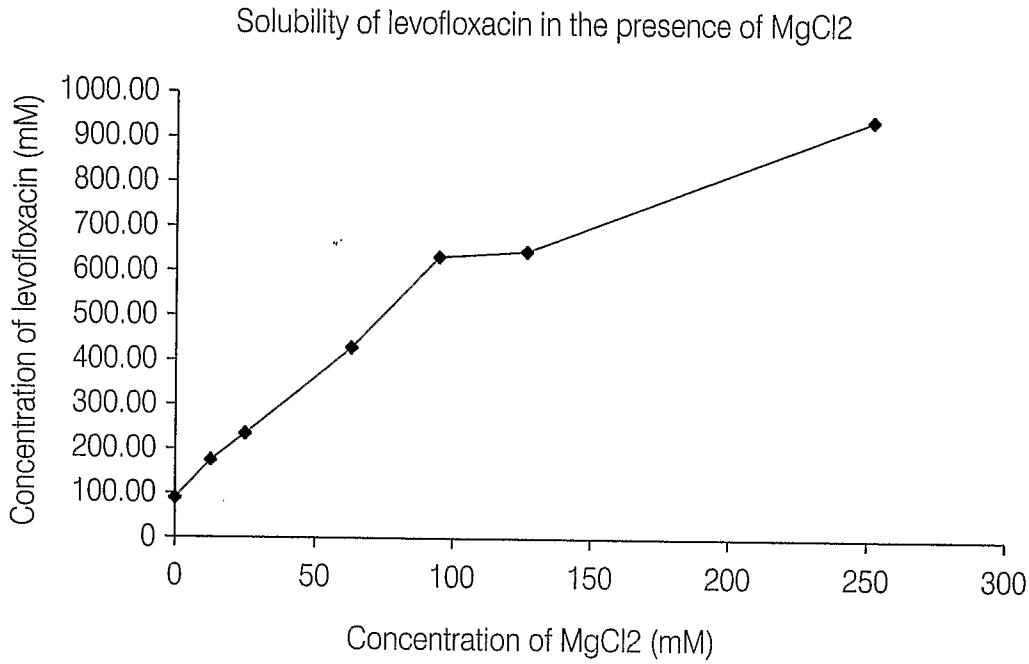

Solubility of levofloxacin in the presence of MgCl2
Concentration of levofloxacin (mM)
1000.00
900.00
800.00
700.00
600.00
500.00
400.00
300.00
200.00
100.00
0
0
50
100
150
200
250
300
Concentration of MgCl2 (mM)

FIG. 50

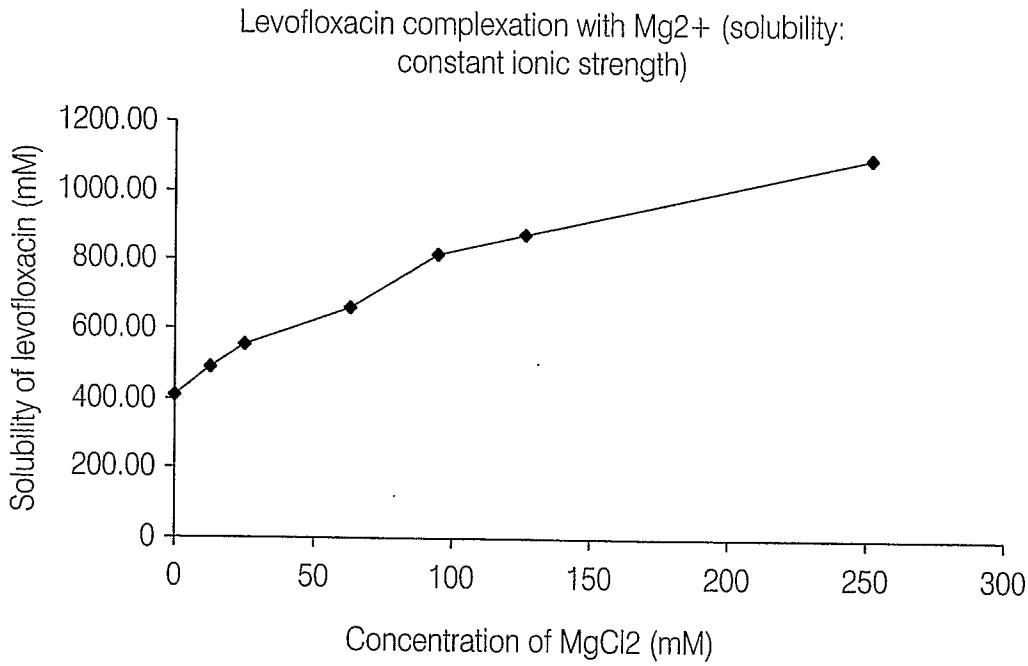

Levofloxacin complexation with Mg2+ (solubility: constant ionic strength)
Solubility of levofloxacin (mM)
1200.00
1000.00
800.00
600.00
400.00
200.00
0
0
50
100
150
200
250
300
Concentration of MgCl2 (mM)

FIG. 51

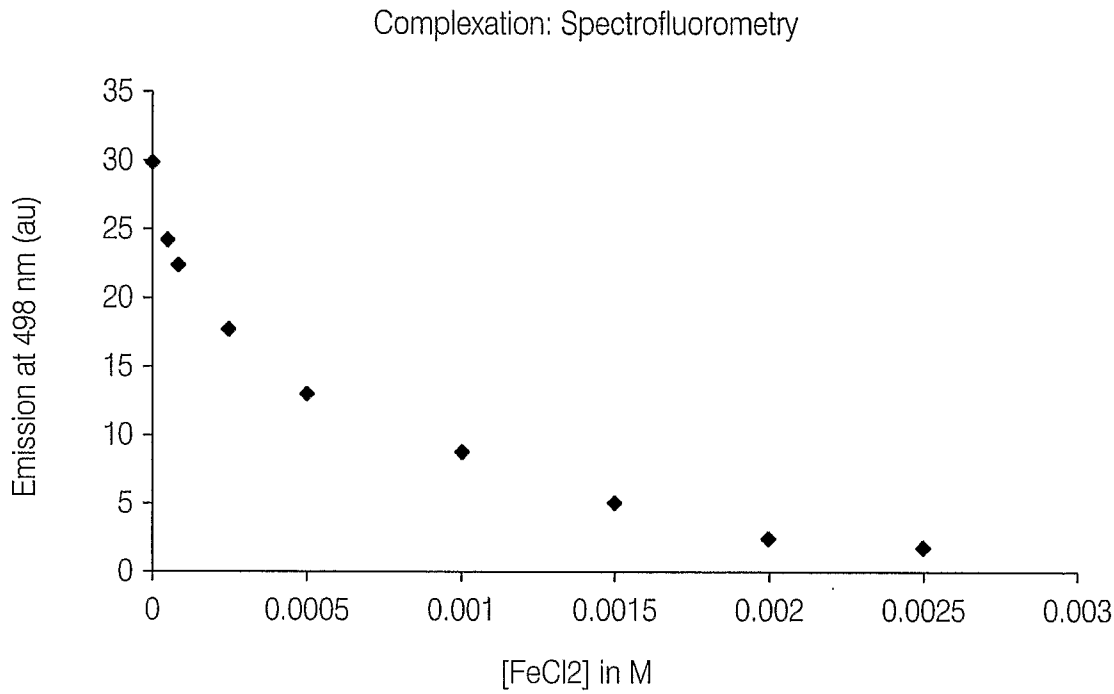

Complexation: Spectrofluorometry
Emission at 498 nm (au)
35
30
25
20
15
10
5
0
0
0.0005
0.001
0.0015
0.002
0.0025
0.003
[FeCl2] in M

FIG. 52

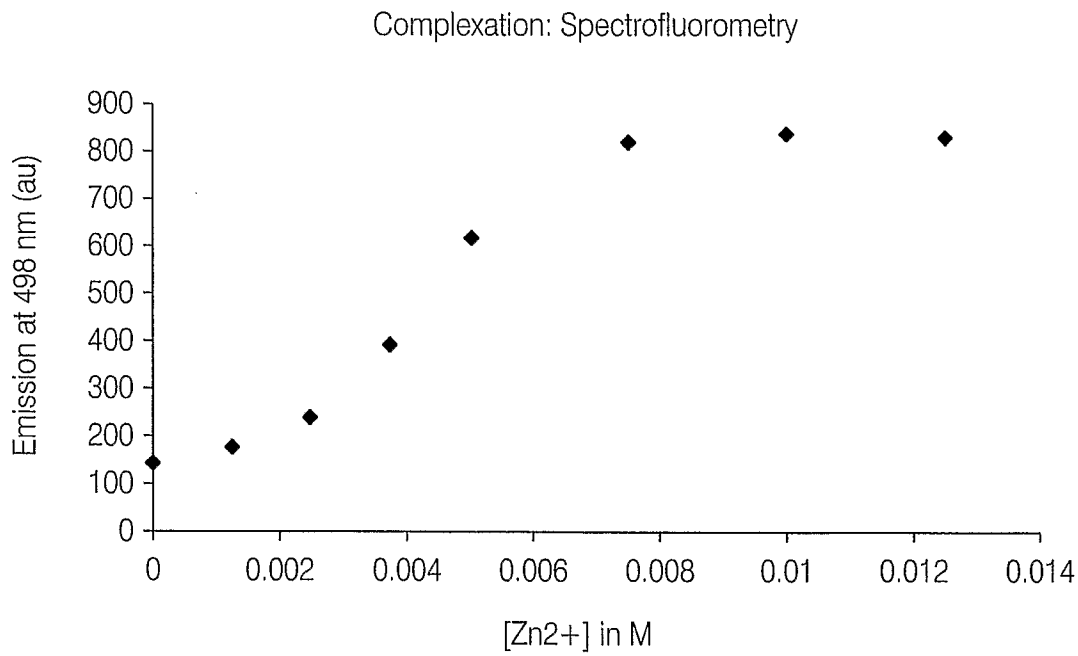

Complexation: Spectrofluorometry
Emission at 498 nm (au)
900
800
700
600
500
400
300
200
100
0
0
0.002
0.004
0.006
0.008
0.01
0.012
0.014
[Zn2+] in M

FIG. 53