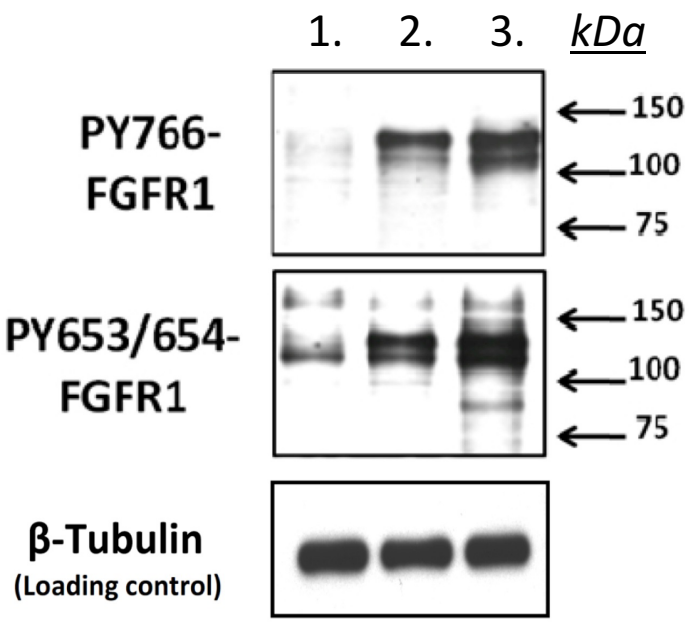
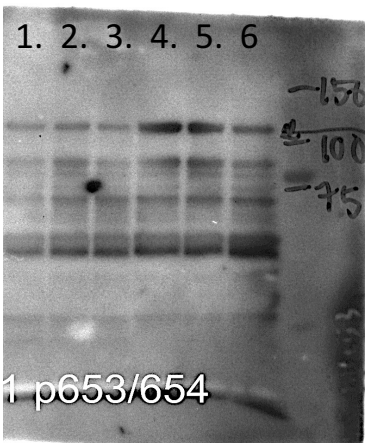
Figure S1 Testing of anti-pY- FGFR1 antibodies
Human endothelial kidney (HEK) 293 cells were transfected or not with a plasmid expressing mouse FGFR1 and stimulated or not with Lo-FGF2 (10 ng/ml, 15 minutes). Subsequently, cell lysates were analyzed by western blotting and probed with antibodies for pY766-FGFR1, pY653/654-FGFR1, or beta (β)-tubulin, as indicated.
Unstransfected cells presented negligible signal for pY766-FGFR1 , and detectable signal for pY653/654-FGFR1. In both cases, the immunoreactive signal increased substantially in transfected cells, especially after stimulation with Lo-FGF2. Intensity of signal for β–tubulin (loading control) appeared similar between the different samples.



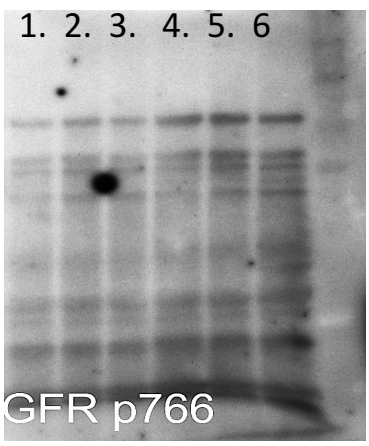
1. Untransfected HEK 293 cells
2. Cells transfected with cDNA for FGFR1
3. Cells transfected with a cDNA for FGFR1 + FGF2

Figure S2. **Lo-FGF2 promotes mito-FGFR1 phosphorylation in hearts**. Western blot staining of cardiac mitochondria (IFM) from saline-perfused (lanes 1,2,3, three preparations) and hearts stimulated with Lo-FGF2 for 15 minutes, lanes 4,5,6, three preparations) for pY-FGFR1. Ponceau S staining indicates similar protein loading between all lanes. A stronger signal for immunoreactive bands (70-110 kDa) can be observed in lanes 4,5 and 6, compared to lanes 1,2 and 3.

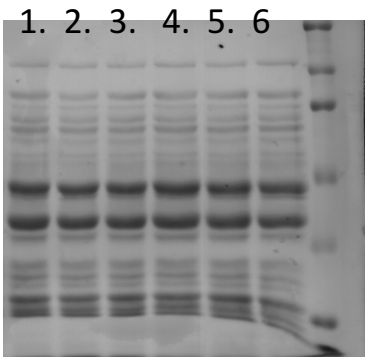
x Anti-pY653/654-FGFR1



x Anti-pY766-FGFR1



Ponceau-S (total protein stain)



Methodology for whole heart perfusion with a Lo-FGF2 containing buffer for 15 minutes, is described in references #17, and #32 (PhD thesis of the first author). Mitochondrial samples (20 microg/lane) were analyzed in 7.5% polyacrylamide gels.

Table S1. The effect of calcium overload, Lo-FGF2, and PKCε inhibitors on cardiac (SSM) mitochondrial Optical Density (OD) at 545 nM

	Control	Lo-FGF2	Lo-FGF2 + eV1-2	Lo-FGF2 + scrambled peptide
Calcium Retention Capacity (nM Ca^{2+} /mg protein)	500 ± 72	936 ± 5.8*	375 ± 56	900 ± 13.5*
Rate of swelling (OD_{545} x 10^3/min)	73.1 ± 5.6	75.5 ± 9.9	61.8 ± 5.4	75.2 ± 6.6
Amplitude of swelling (% of maximal swelling)	30.0 ± 0.3	29.3 ± 2.5	29.6 ± 2.3	32.9 ± 3.3

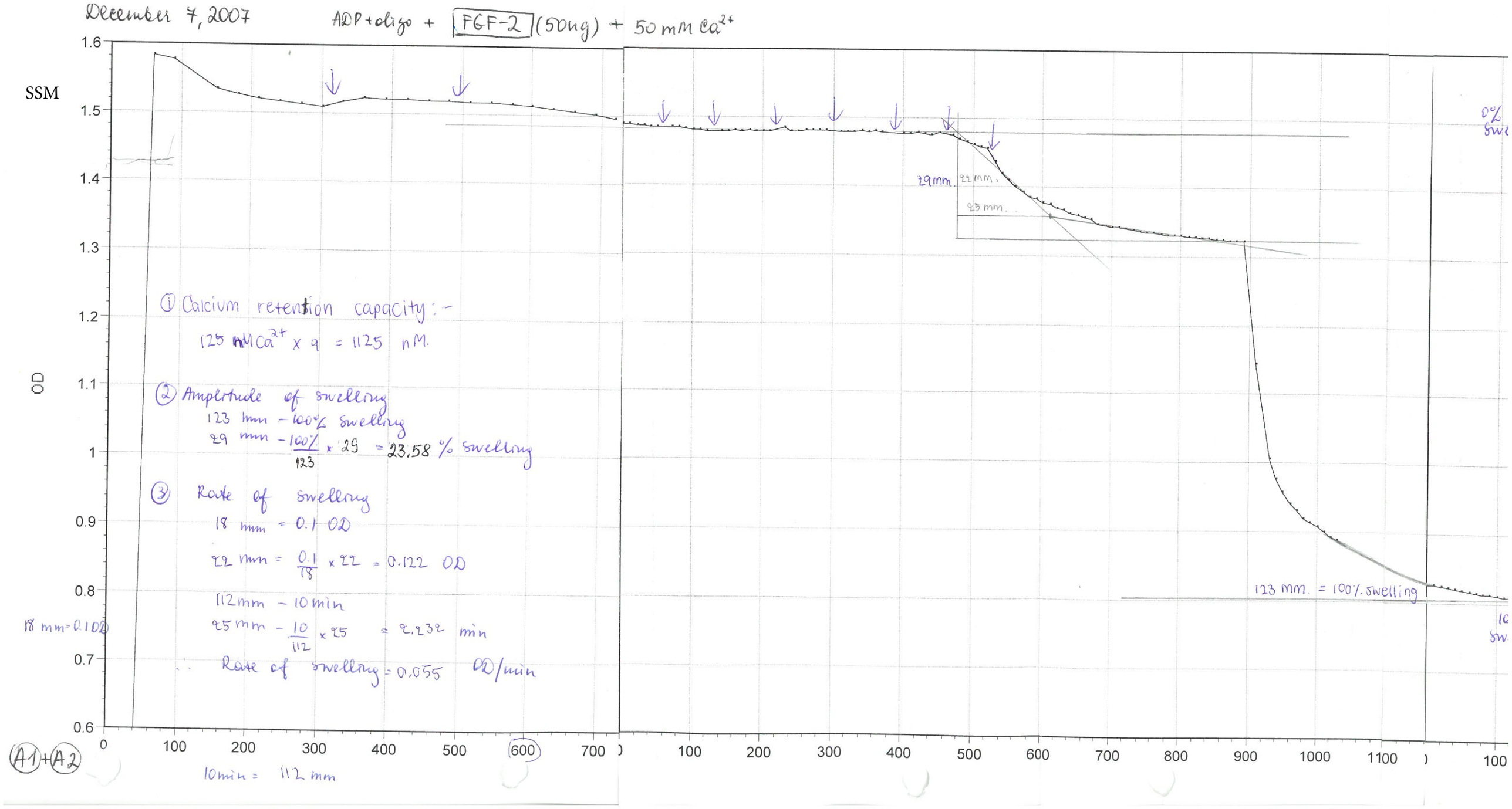
Table S2. The effect of Lo-FGF2 on cardiac mitochondria (SSM) Respiration

	Rate of Respiration		RCI (State 3/ State 4)	ADP/O ratio nmol ADP/ nmol O2	Rate of Oxidative Phosphorylation (nmol ATP/min/ mg protein)
	State 3	State 4			
Control	154.3 ± 6.7	19.9 ± 1.2	7.79 ± 0.26	2.51 ± 0.05	387.3 ± 19.5
Lo-FGF2	171.3 ± 10.3	20.0 ± 2.4	8.85 ± 0.87	2.56 ± 0.04	437.9 ± 25.8

December 7, 2007

ADP + oligo + FGF-2 (50ng) + 50 mM Ca^{2+}

SSM



① Calcium retention capacity :-

125 mM Ca^{2+} x 9 = 1125 nM.

② Amplitude of swelling

123 mm - 100% swelling

29 mm - $\frac{100\%}{123} \times 29$ = 23.58 % swelling

③ Rate of swelling

18 mm = 0.1 OD

22 mm = $\frac{0.1}{18} \times 22$ = 0.122 OD

112 mm - 10 min

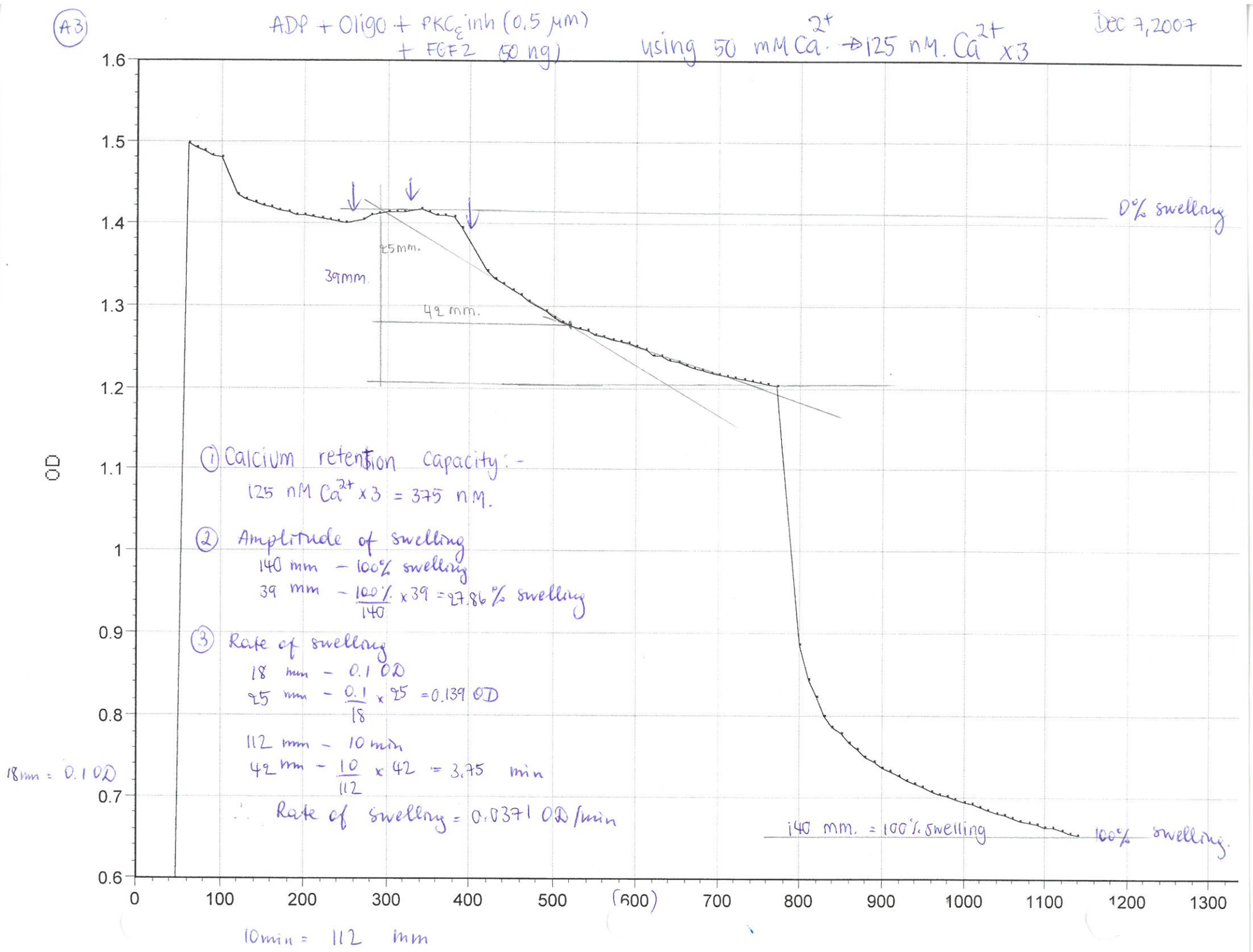
25 mm - $\frac{10}{112} \times 25$ = 2.232 min

∴ Rate of swelling = 0.055 OD/min

(A1)+(A2)

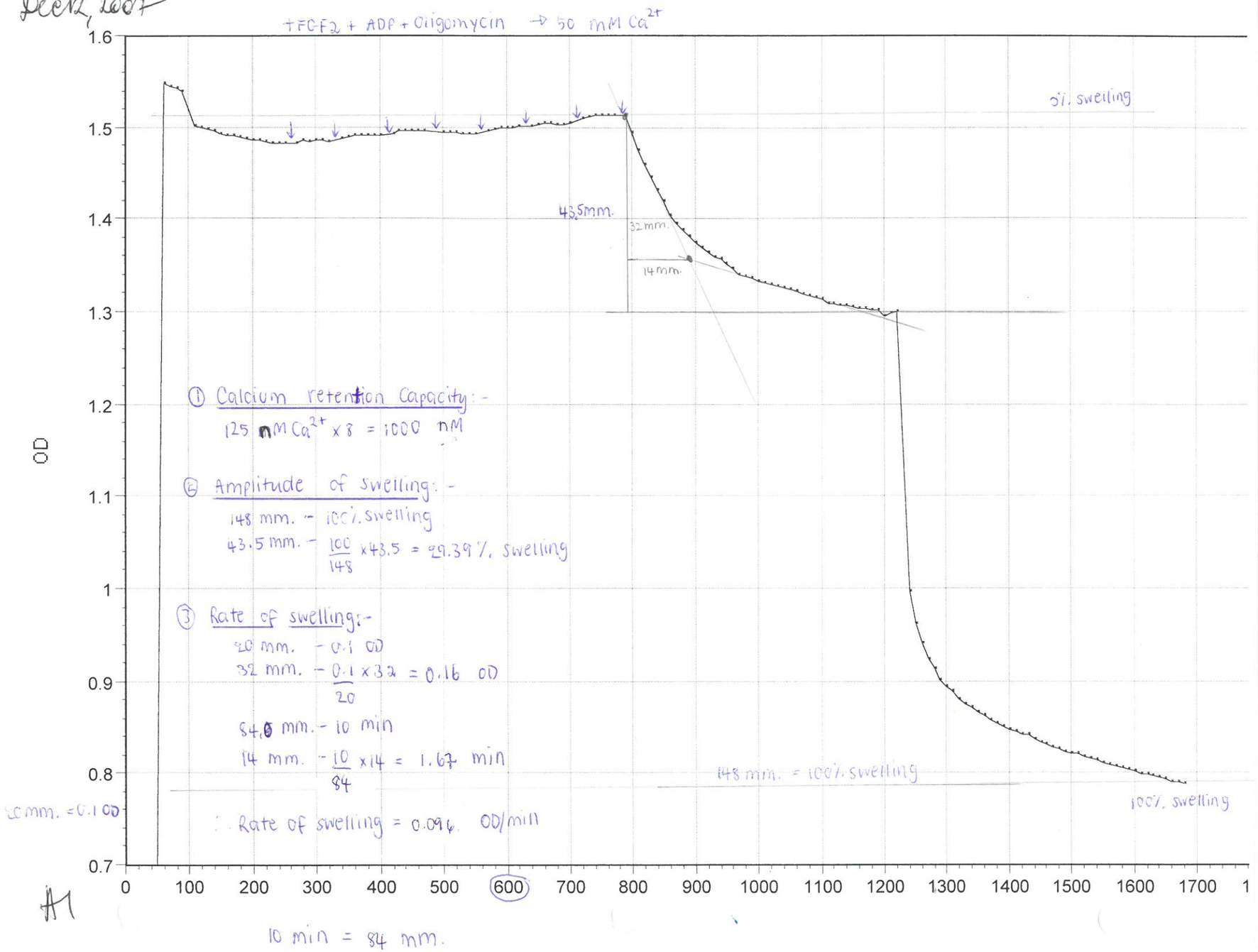
10 min = 112 mm

SSM


A3
ADP + Oligo + PKCε inh (0.5 μM)
+ FGF2 (50 ng)
using 50 mM Ca2+ → 125 nM. Ca2+ x3
Dec 7, 2007
OD
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
0% swelling
25mm.
39mm.
42 mm.
① Calcium retention capacity:-
125 nM Ca2+ x 3 = 375 nM.
② Amplitude of swelling
140 mm – 100% swelling
39 mm – 100%/140 x 39 = 27.86% swelling
③ Rate of swelling
18 mm – 0.1 OD
25 mm – 0.1/18 x 25 = 0.139 OD
112 mm – 10 min
42 mm – 10/112 x 42 = 3.75 min
∴ Rate of swelling = 0.0371 OD/min
18mm = 0.1 OD
140 mm. = 100% swelling
100% swelling.
10min = 112 mm

Dec 12, 2007

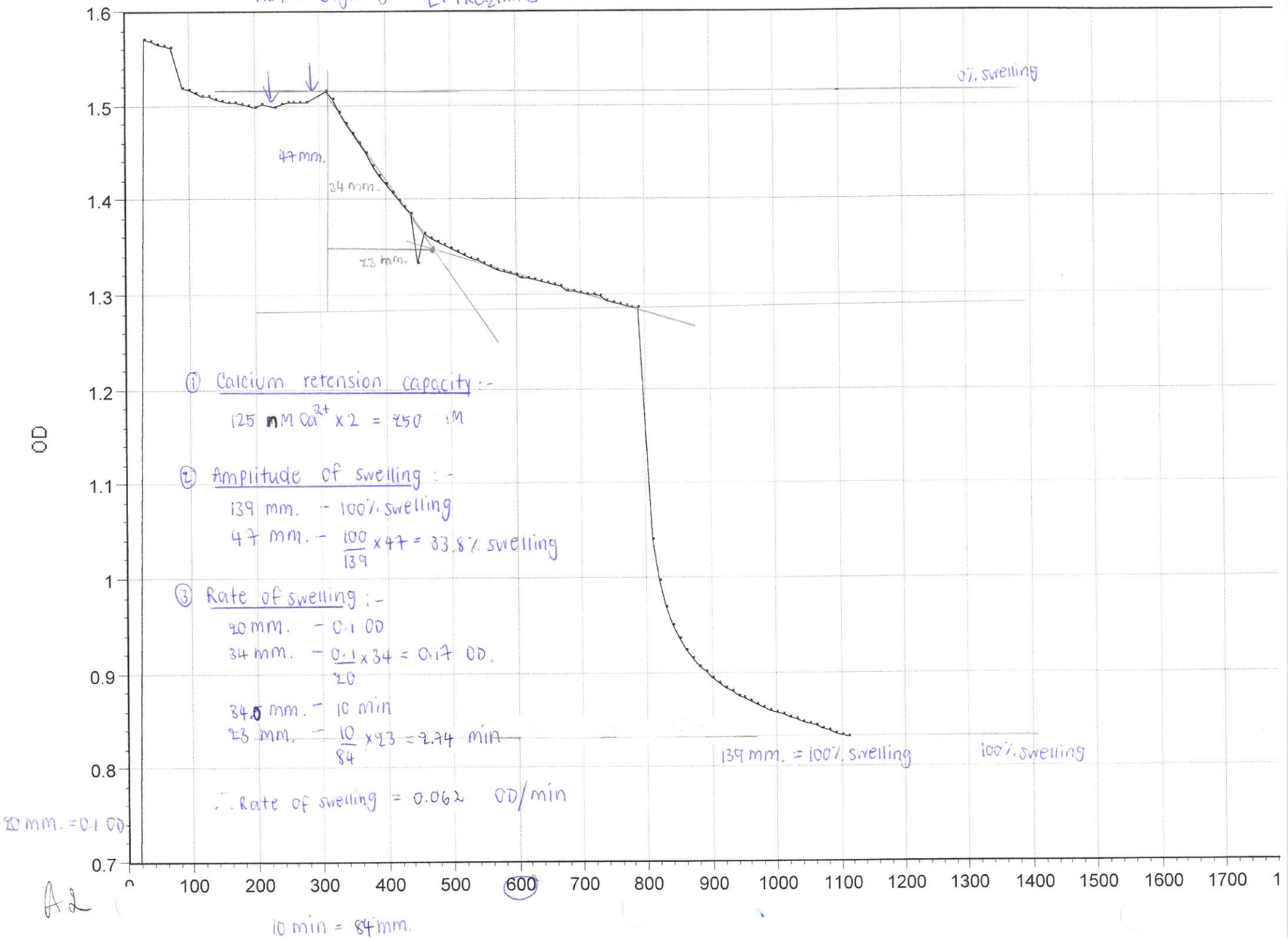
SSM



SSM

Dec 12, 2007

ADP + Oligomycin [+FGF2 / + PKC$_\varepsilon$ inh.] → 50 mM Ca^{2+}



① Calcium retension capacity :-

125 mM Ca^{2+} x 2 = 250 μM

② Amplitude of swelling :-

139 mm. - 100% swelling

47 mm. - $\frac{100}{139}$ x 47 = 33.8% swelling

③ Rate of swelling :-

20 mm. - 0.1 OD

34 mm. - $\frac{0.1}{20}$ x 34 = 0.17 OD.

84 mm. - 10 min

23 mm. - $\frac{10}{84}$ x 23 = 2.74 min

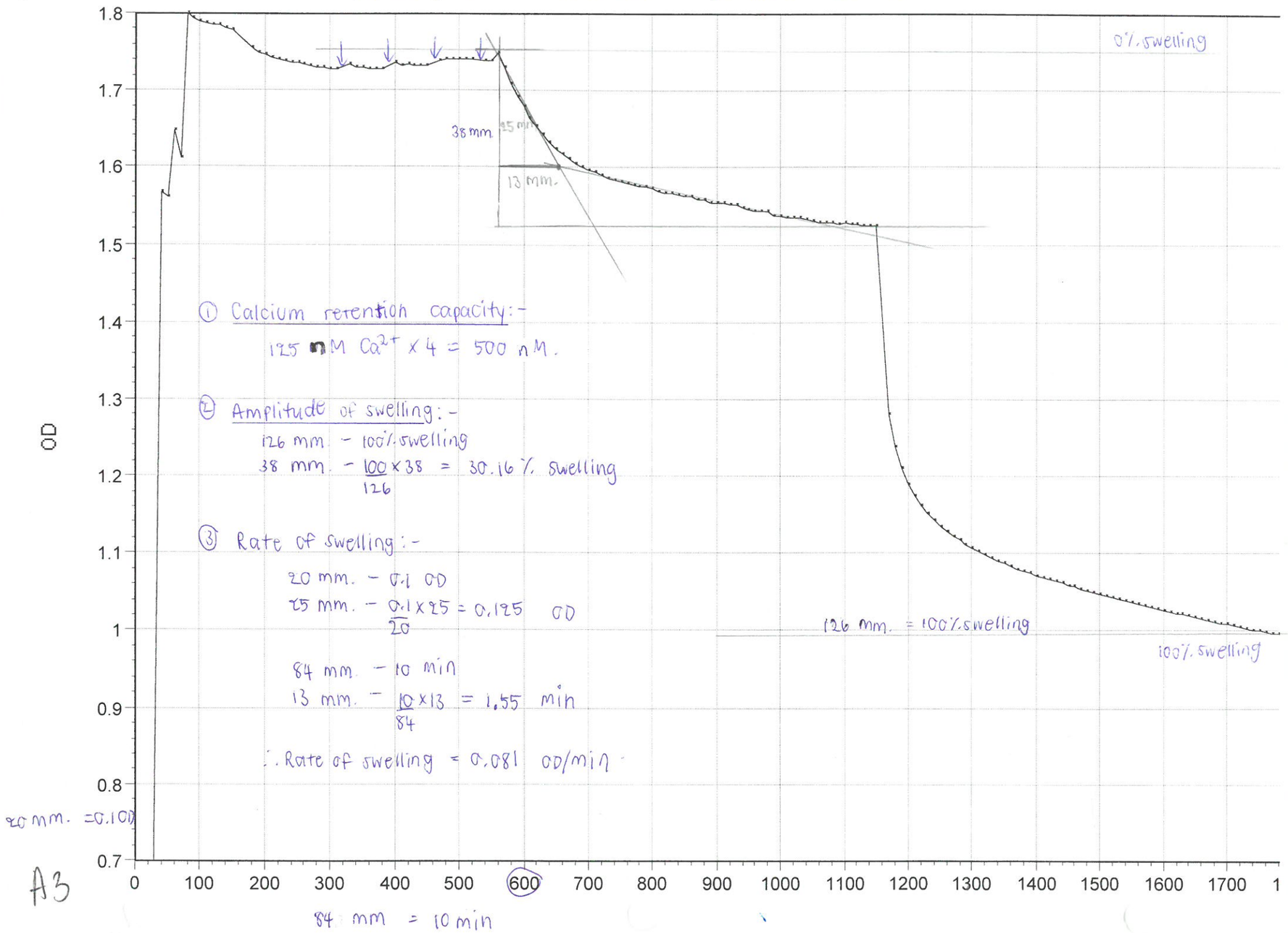
∴ Rate of swelling = 0.062 OD/min

A2

SSM

Dec 12, 2007

ADP + [illegible]mycin. → 50 mM Ca^{2+} NO FOF2 + NO PKC_ε inh.



① Calcium retention capacity:-

125 nM Ca^{2+} × 4 = 500 nM.

② Amplitude of swelling:-

126 mm. - 100% swelling

38 mm. - $\frac{100}{126} \times 38$ = 30.16 % swelling

③ Rate of swelling:-

20 mm. - 0.1 OD

25 mm. - $\frac{0.1}{20} \times 25 = 0.125$ OD

84 mm - 10 min

13 mm. - $\frac{10}{84} \times 13 = 1.55$ min

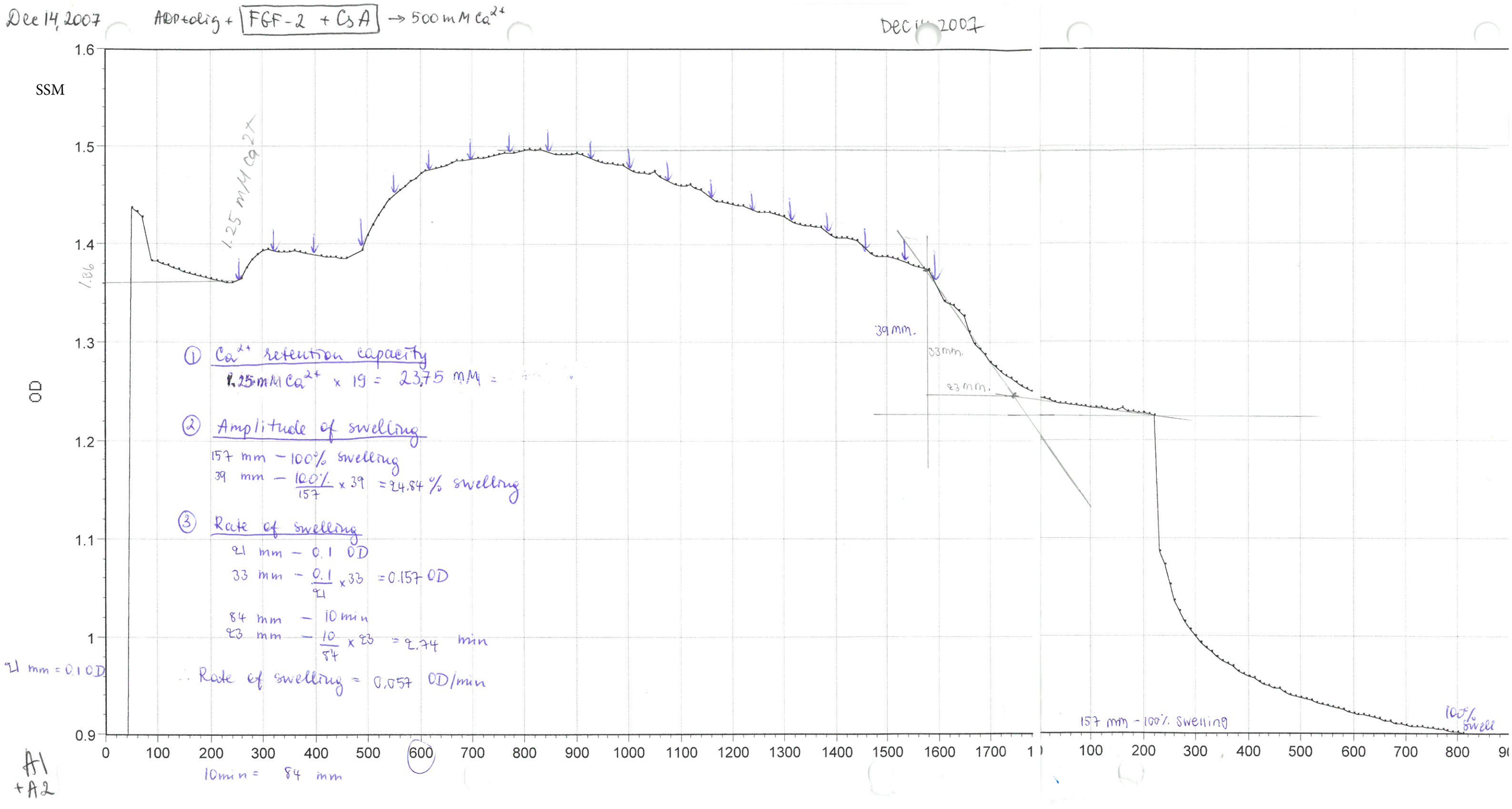
∴ Rate of swelling = 0.081 OD/min

A3

Dec 14, 2007 ADP+olig + FGF-2 + CsA → 500 mM Ca^{2+}

Dec 14, 2007

SSM



① Ca^{2+} retention capacity

1.25 mM Ca^{2+} x 19 = 23.75 mM

② Amplitude of swelling

157 mm – 100% swelling

39 mm – $\frac{100\%}{157} \times 39$ = 24.84 % swelling

③ Rate of swelling

21 mm – 0.1 OD

33 mm – $\frac{0.1}{21} \times 33$ = 0.157 OD

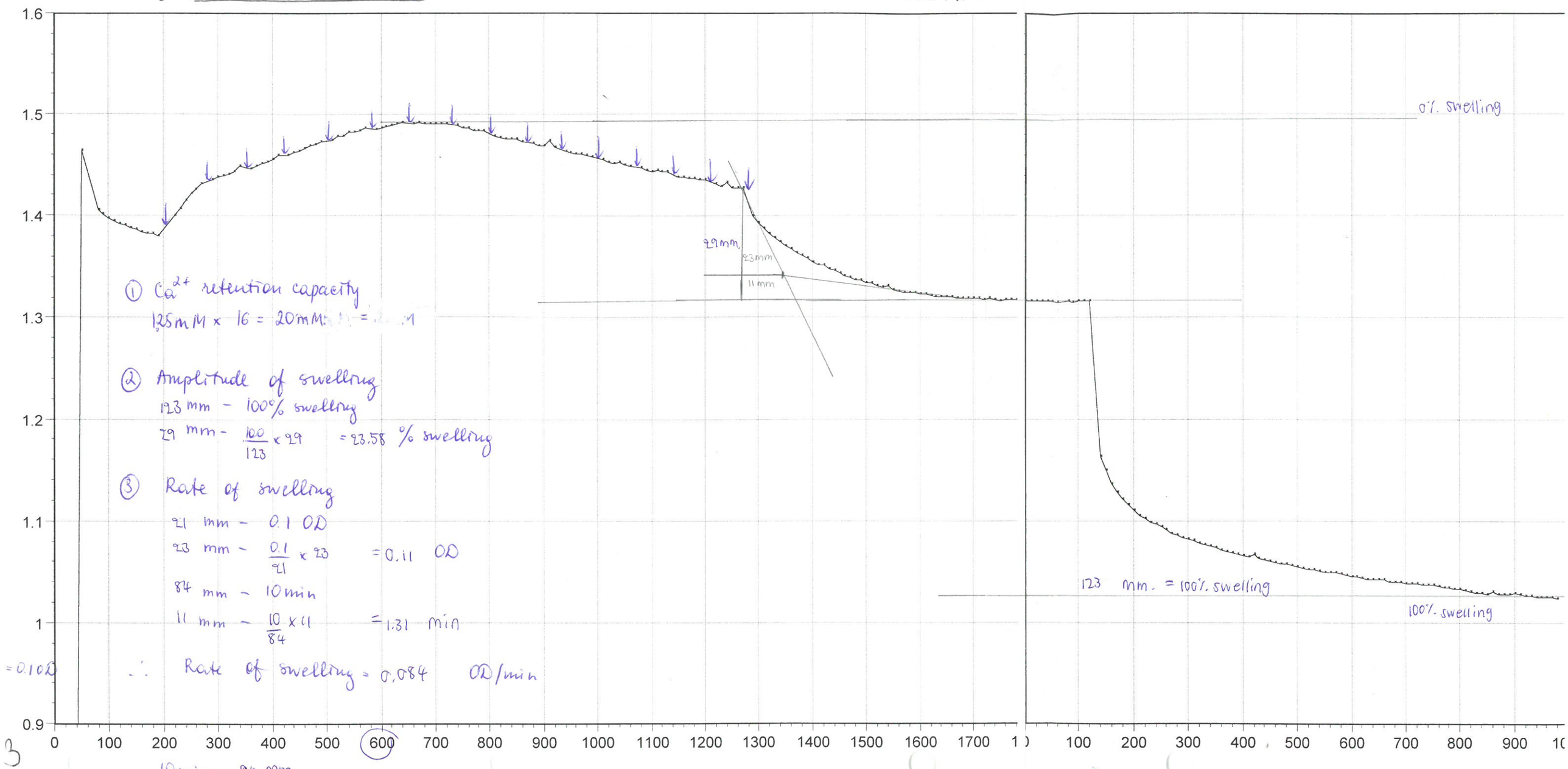
84 mm – 10 min

83 mm – $\frac{10}{84} \times 83$ = 2.74 min

∴ Rate of swelling = 0.057 OD/min

A1 +A2

1, 2007 ADP+alig + FGF-2+ PKCε Inhib + CsA → 500mM Ca^{2+} SSM Dec 14, 2007



① Ca^{2+} retention capacity

125mM x 16 = 20mM: M = .M

② Amplitude of swelling

123 mm – 100% swelling

29 mm – $\frac{100}{123} \times 29$ = 23.58 % swelling

③ Rate of swelling

21 mm – 0.1 OD

23 mm – $\frac{0.1}{21} \times 23$ = 0.11 OD

84 mm – 10 min

11 mm – $\frac{10}{84} \times 11$ = 1.31 min

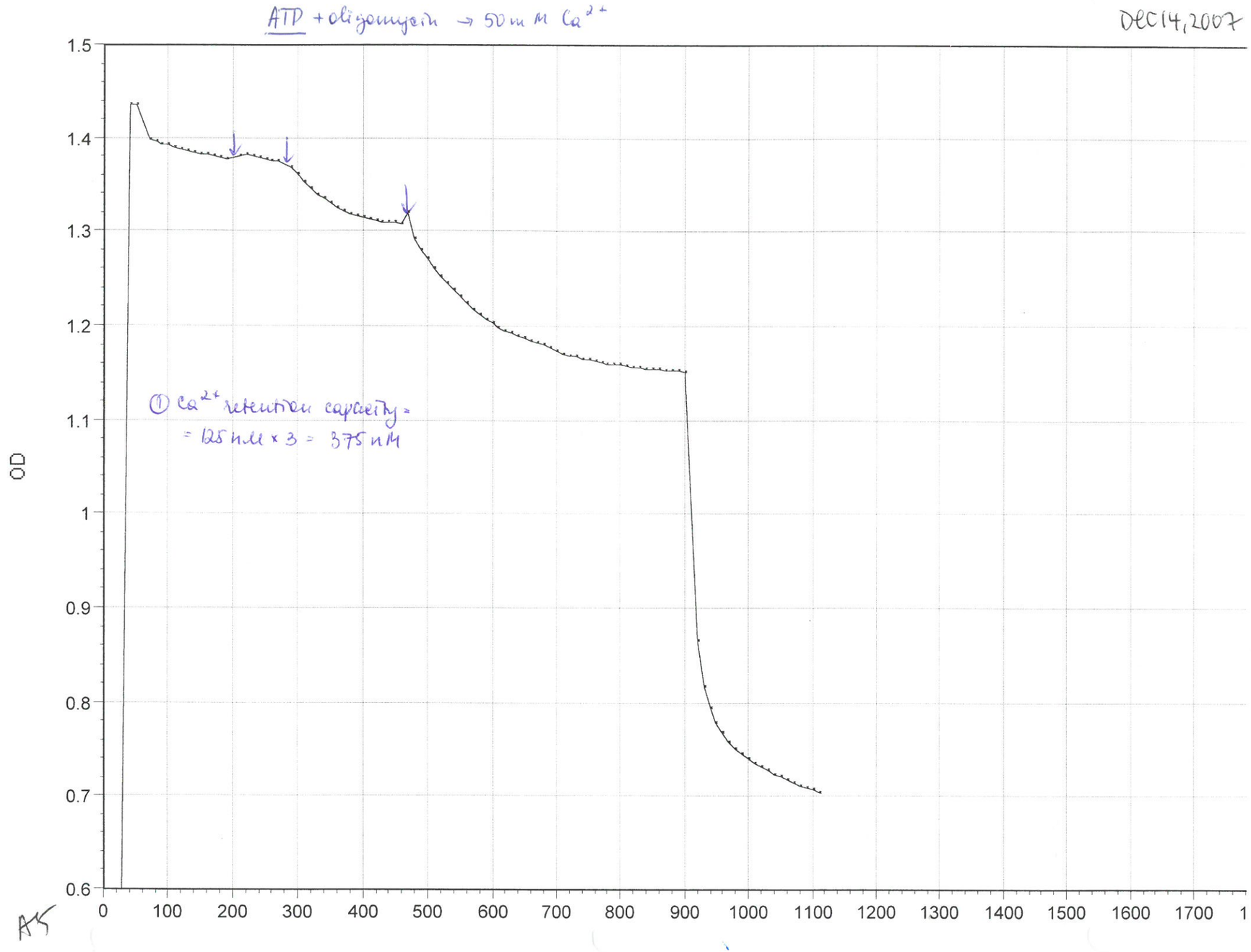
∴ Rate of swelling = 0.084 OD/min

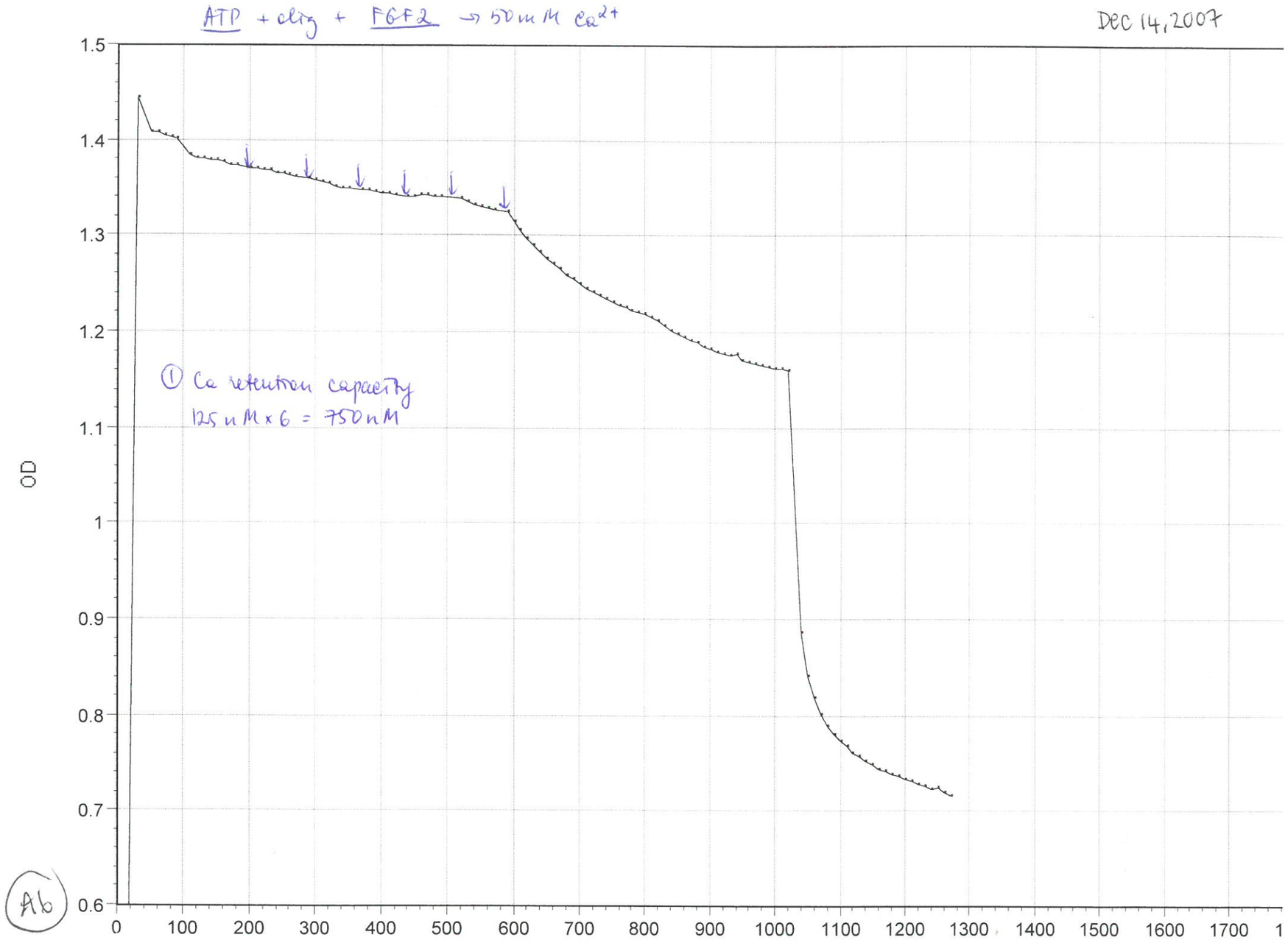
=0.10

3

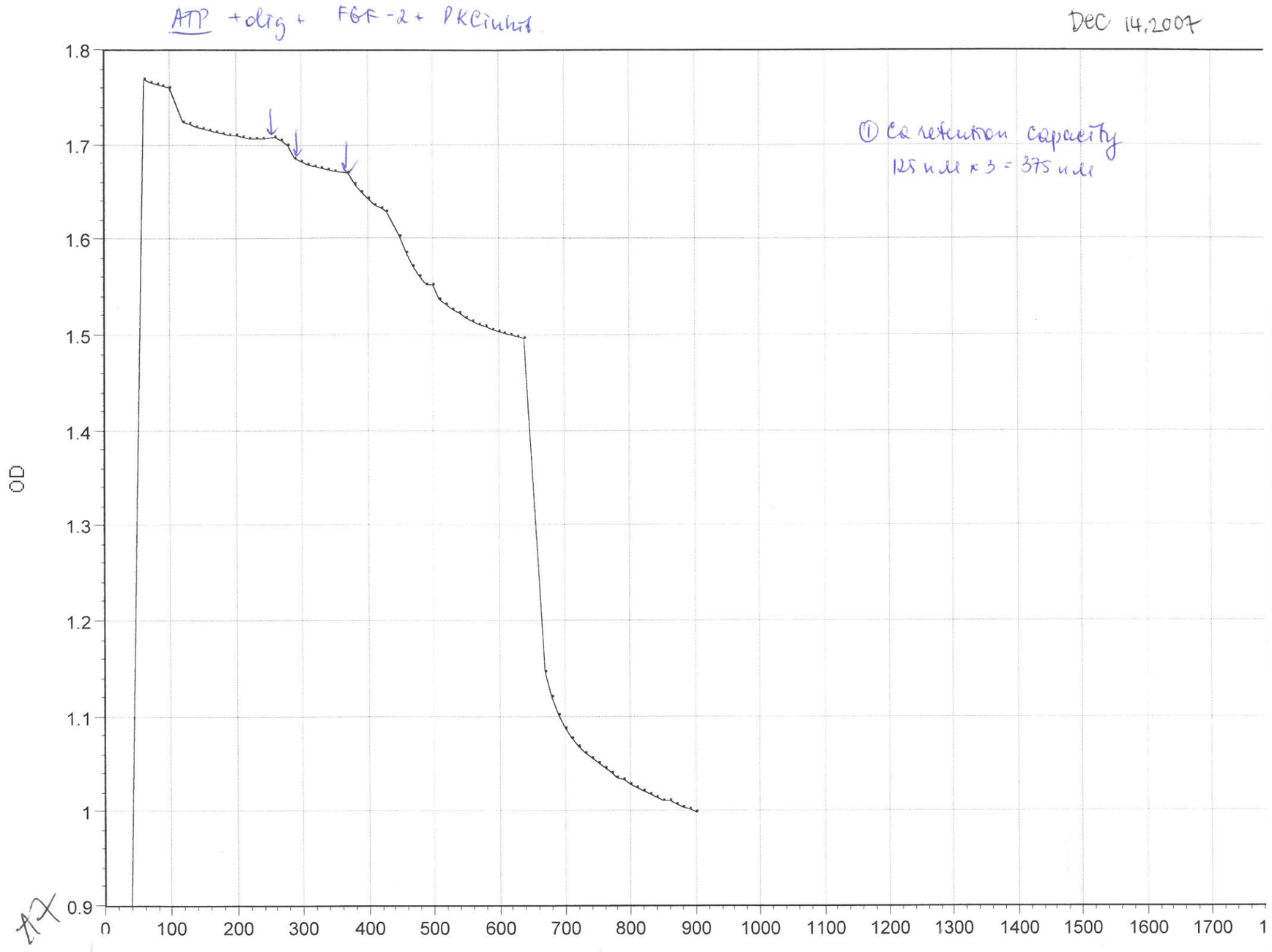
10 min = 84 mm.

SSM

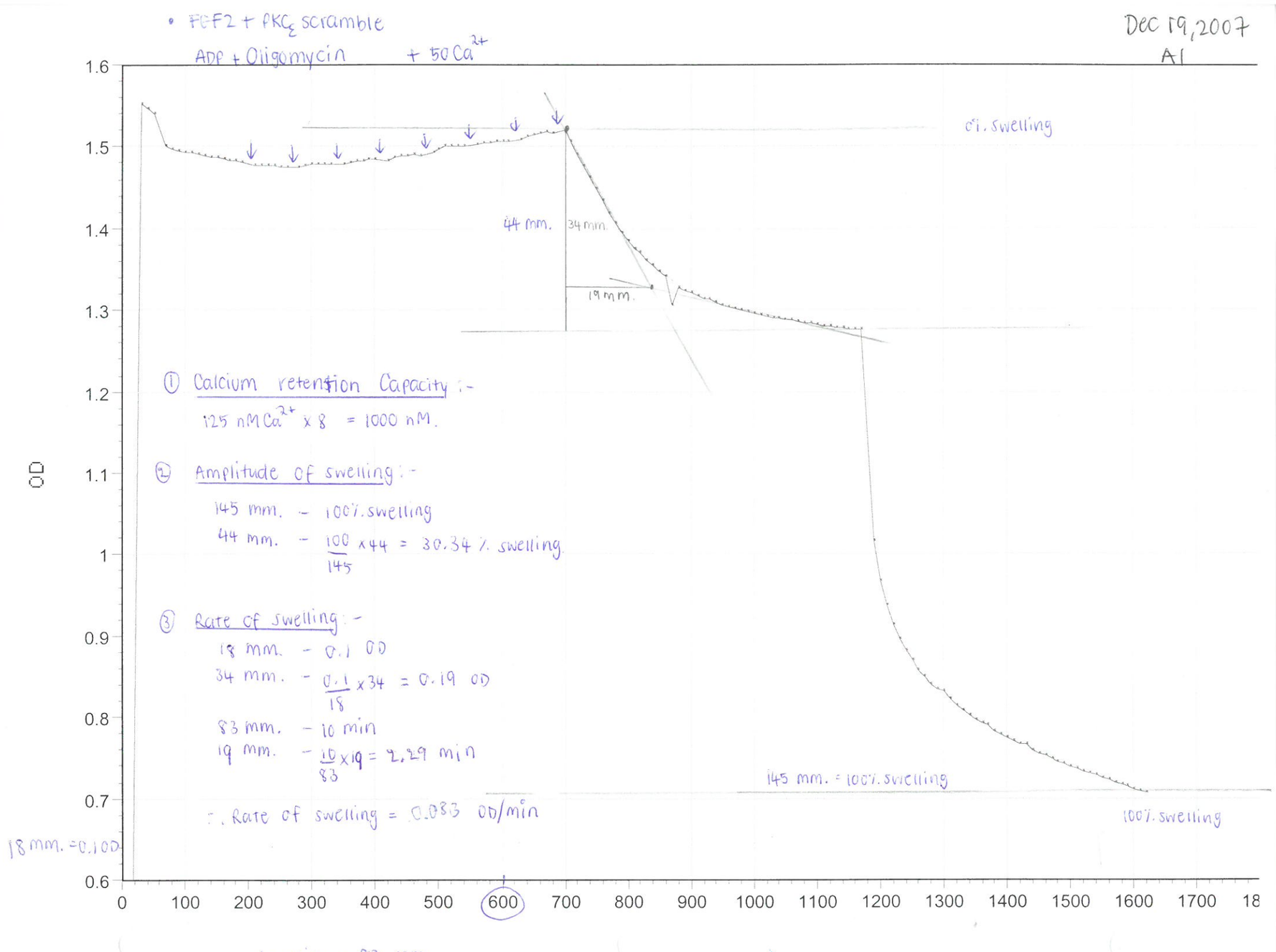

ATP + oligomycin → 50 mM Ca2+
DEC14,2007
OD
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
① Ca2+ retention capacity =
= 125 nM × 3 = 375 nM
AK


ATP + clrg + FGF2 → 50 m M Ca2+
Dec 14, 2007
OD
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
① Ca retention capacity
125 nM x 6 = 750 nM
Ab

SSM


ATP +drug + FGF-2 + PKCinhib
Dec 14,2007
① Ca retention capacity
125 nM x 3 = 375 nM
OD
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1

SSM


• FGF2 + PKCε scramble
ADP + Oligomycin + 50 Ca2+
Dec 19, 2007
A1
OD
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
0% swelling
44 mm.
34 mm.
19 mm.
① Calcium retention Capacity :-
125 nM Ca2+ x 8 = 1000 nM.
② Amplitude of swelling :-
145 mm. - 100% swelling
44 mm. - 100/145 x 44 = 30.34 % swelling
③ Rate of swelling :-
18 mm. - 0.1 OD
34 mm. - 0.1/18 x 34 = 0.19 OD
83 mm. - 10 min
19 mm. - 10/83 x 19 = 2.29 min
∴ Rate of swelling = 0.083 OD/min
145 mm. = 100% swelling
100% swelling
18 mm. = 0.1 OD
10 min = 83 mm.

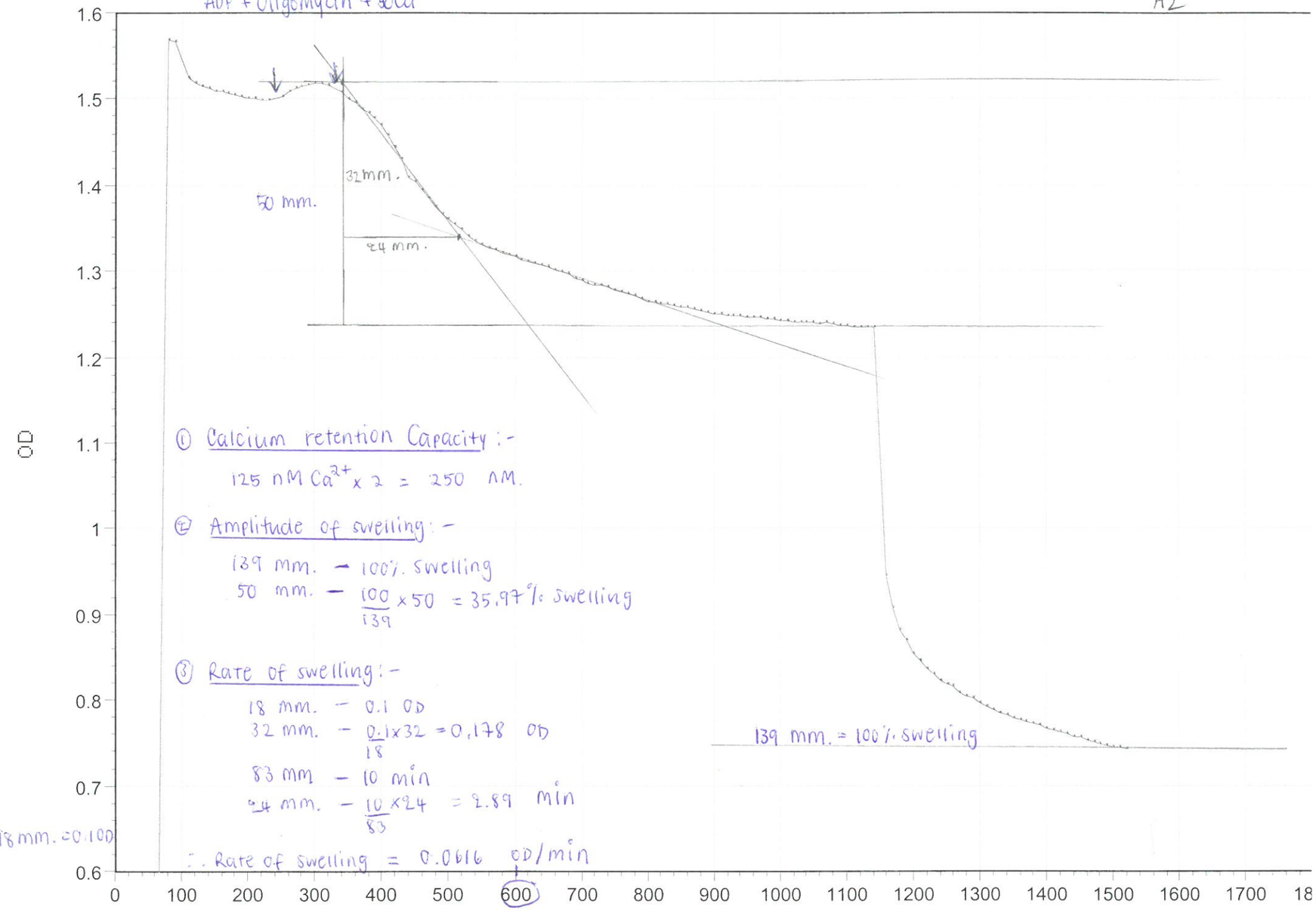
SSM

• FGF2 + PKCε inhibitor

ADP + Oligomycin + Ca^{2+}

Dec 19, 2007

A2



① Calcium retention Capacity :-

125 nM Ca^{2+} x 2 = 250 nM.

② Amplitude of swelling :-

139 mm. — 100% swelling

50 mm. — $\frac{100}{139} \times 50 = 35.97\%$ swelling

③ Rate of swelling :-

18 mm. — 0.1 OD

32 mm. — $\frac{0.1 \times 32}{18} = 0.178$ OD

83 mm — 10 min

24 mm. — $\frac{10}{83} \times 24 = 2.89$ min

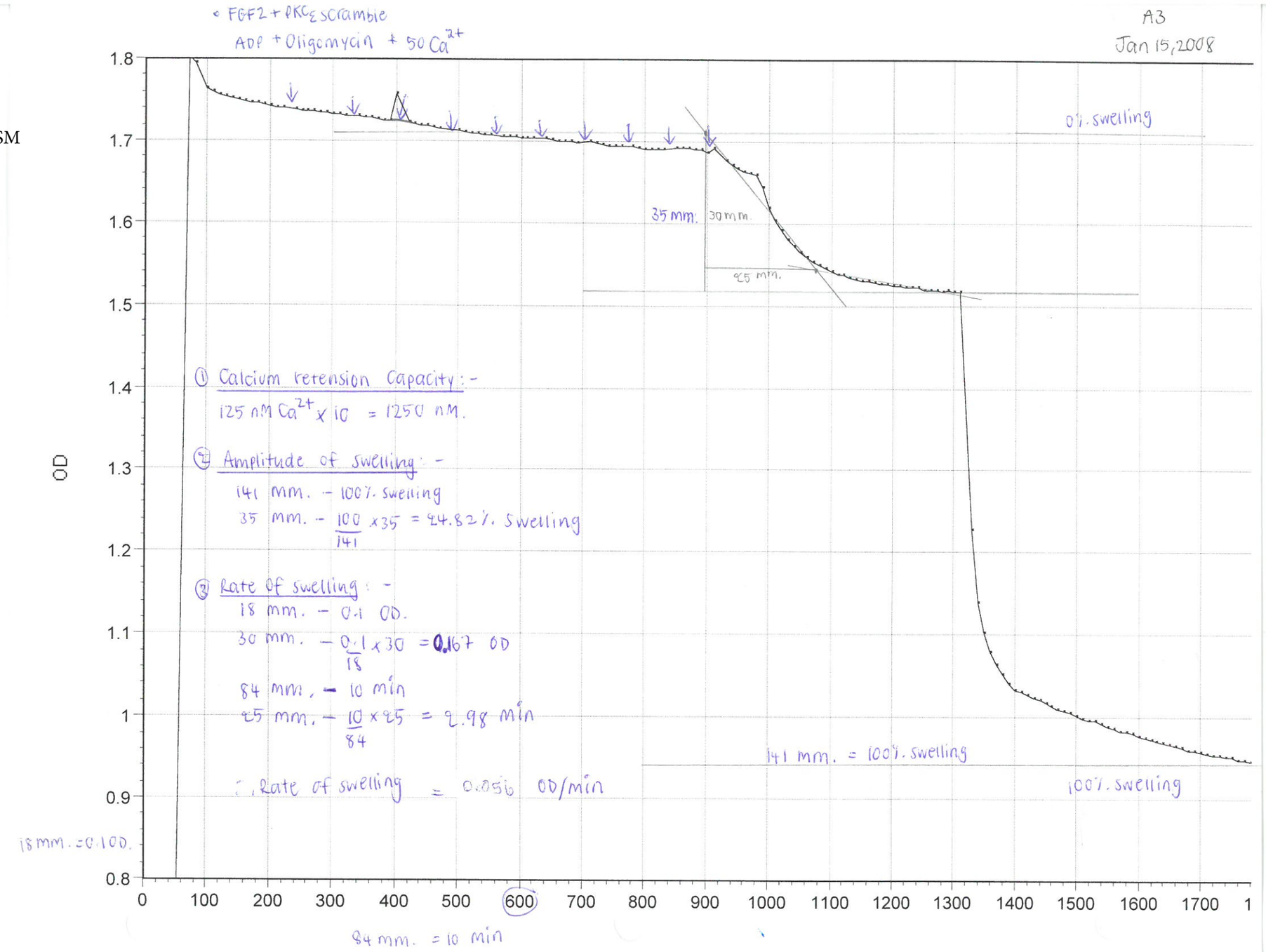
∴ Rate of swelling = 0.0616 OD/min

10 min = 83 mm.

SSM

A3

Jan 15, 2008

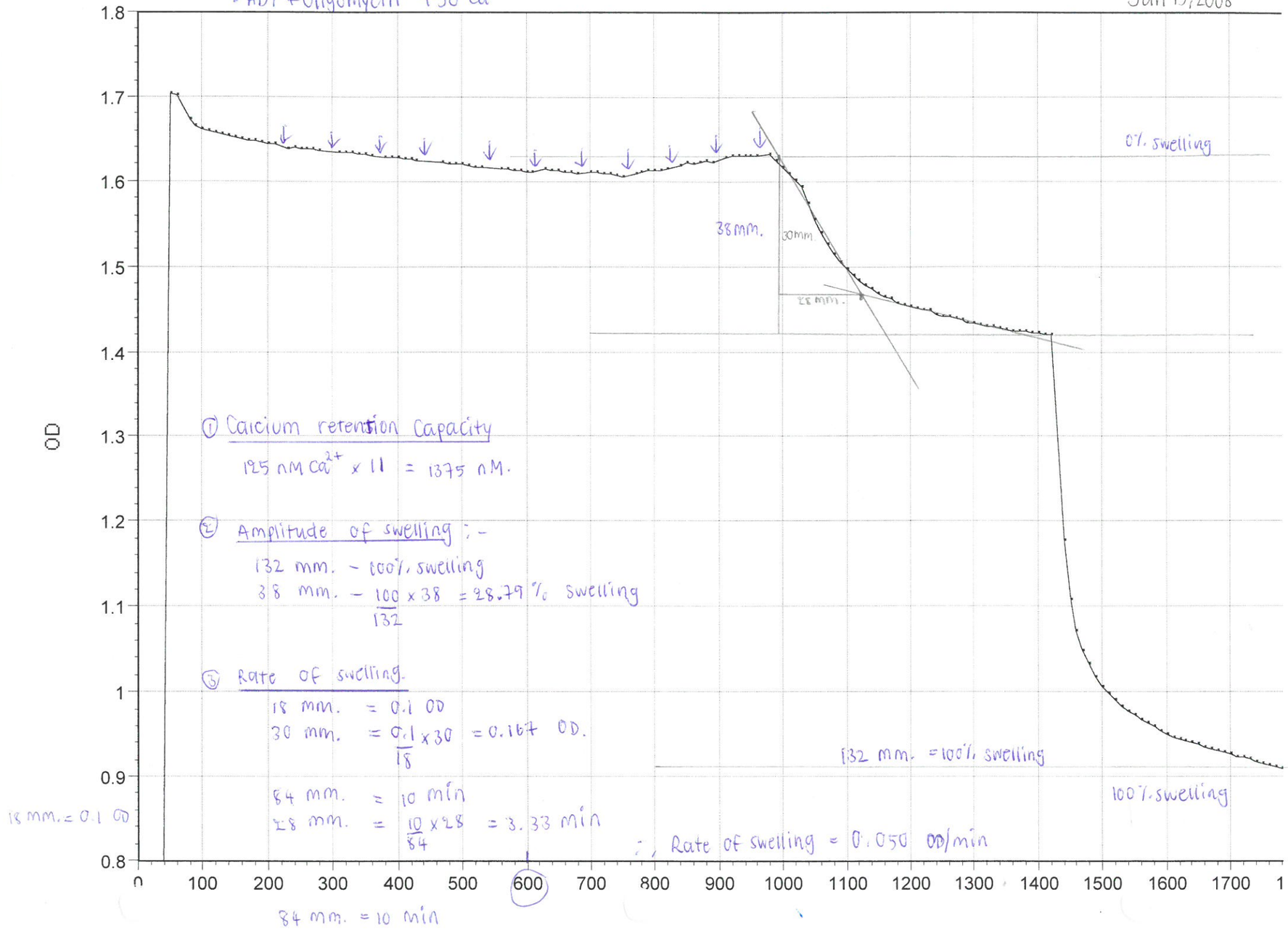


SSM

A1

Jan 15, 2008

- ~~FOF2~~
- ADP + Oligomycin + 50 Ca^{2+}



① Calcium retention Capacity

125 nM Ca^{2+} x 11 = 1375 nM.

② Amplitude of swelling :-

132 mm. - 100% swelling

38 mm. - $\frac{100}{132} \times 38$ = 28.79 % swelling

③ Rate of swelling.

18 mm. = 0.1 OD

30 mm. = $\frac{0.1}{18} \times 30$ = 0.167 OD.

84 mm. = 10 min

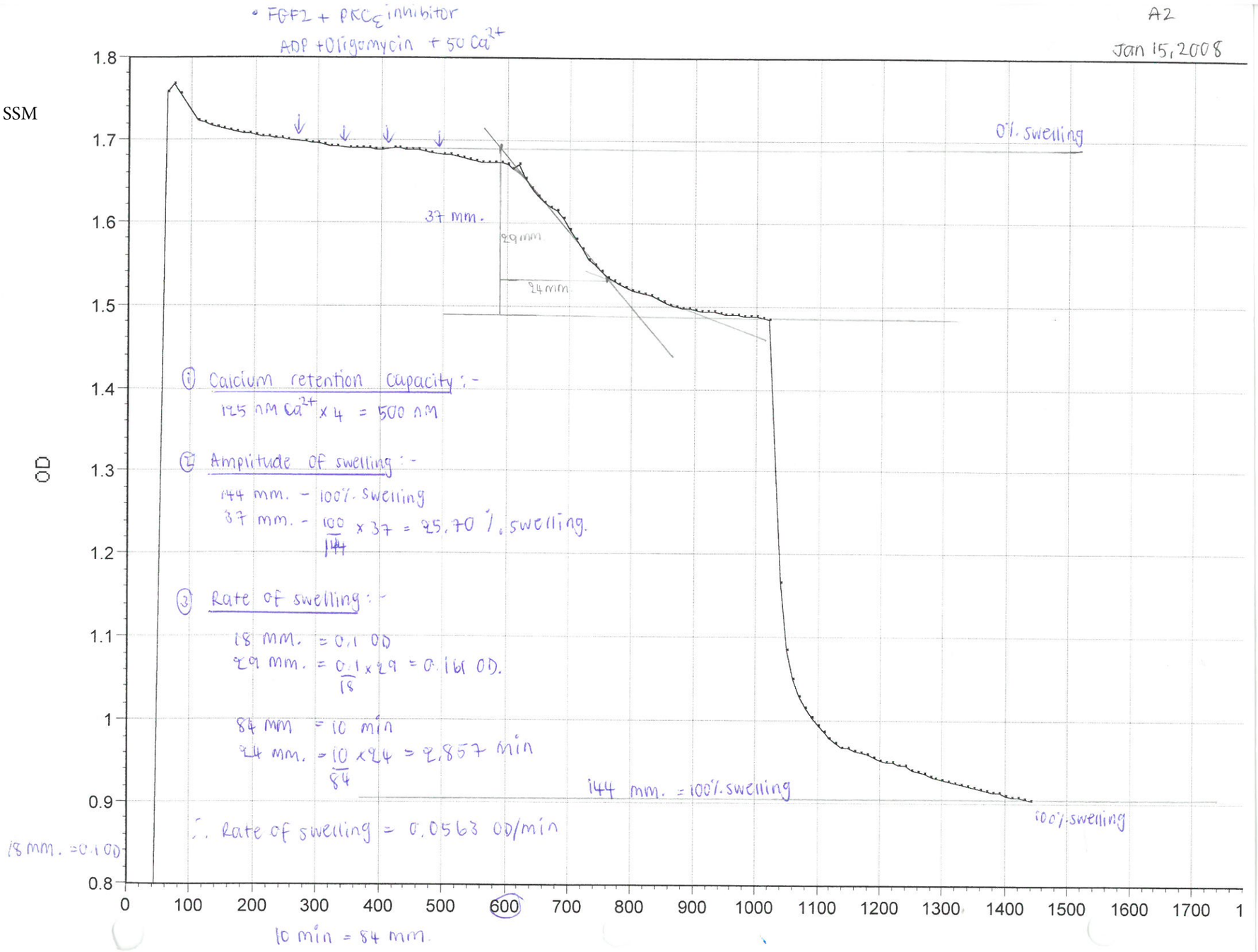
28 mm. = $\frac{10}{84} \times 28$ = 3.33 min

∴ Rate of swelling = 0.050 OD/min

SSM

A2

Jan 15, 2008

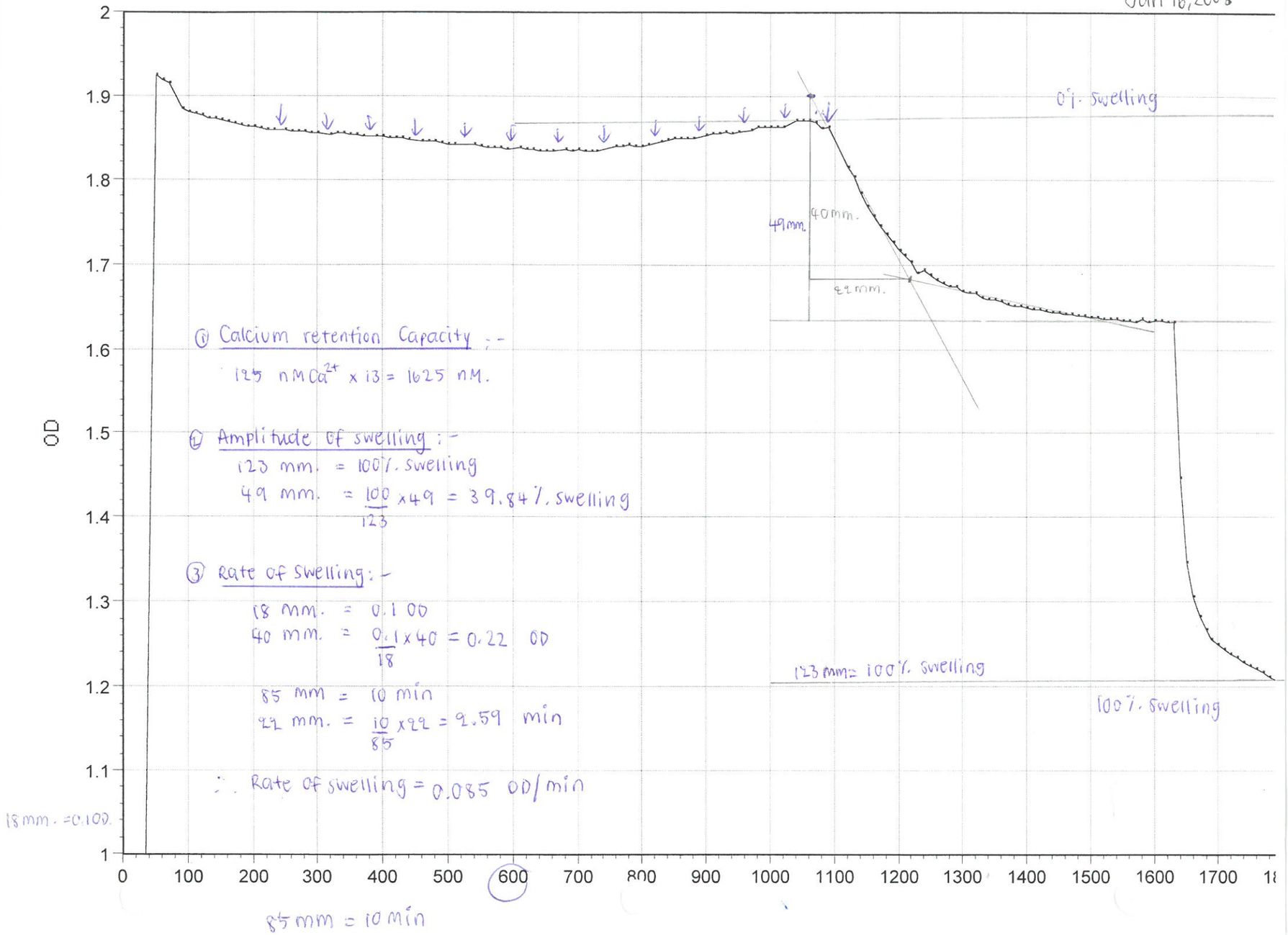


SSM

*FGF2 + PKC$_\varepsilon$ scrambie

A1

Jan 16, 2008



① Calcium retention Capacity :-

125 nM Ca^{2+} x 13 = 1625 nM.

② Amplitude of swelling :-

123 mm. = 100% swelling

49 mm. = $\frac{100}{123} \times 49 = 39.84\%$ swelling

③ Rate of swelling :-

18 mm. = 0.100D

40 mm. = $\frac{0.1}{18} \times 40 = 0.22$ OD

85 mm = 10 min

22 mm. = $\frac{10}{85} \times 22 = 2.59$ min

∴ Rate of swelling = 0.085 OD/min

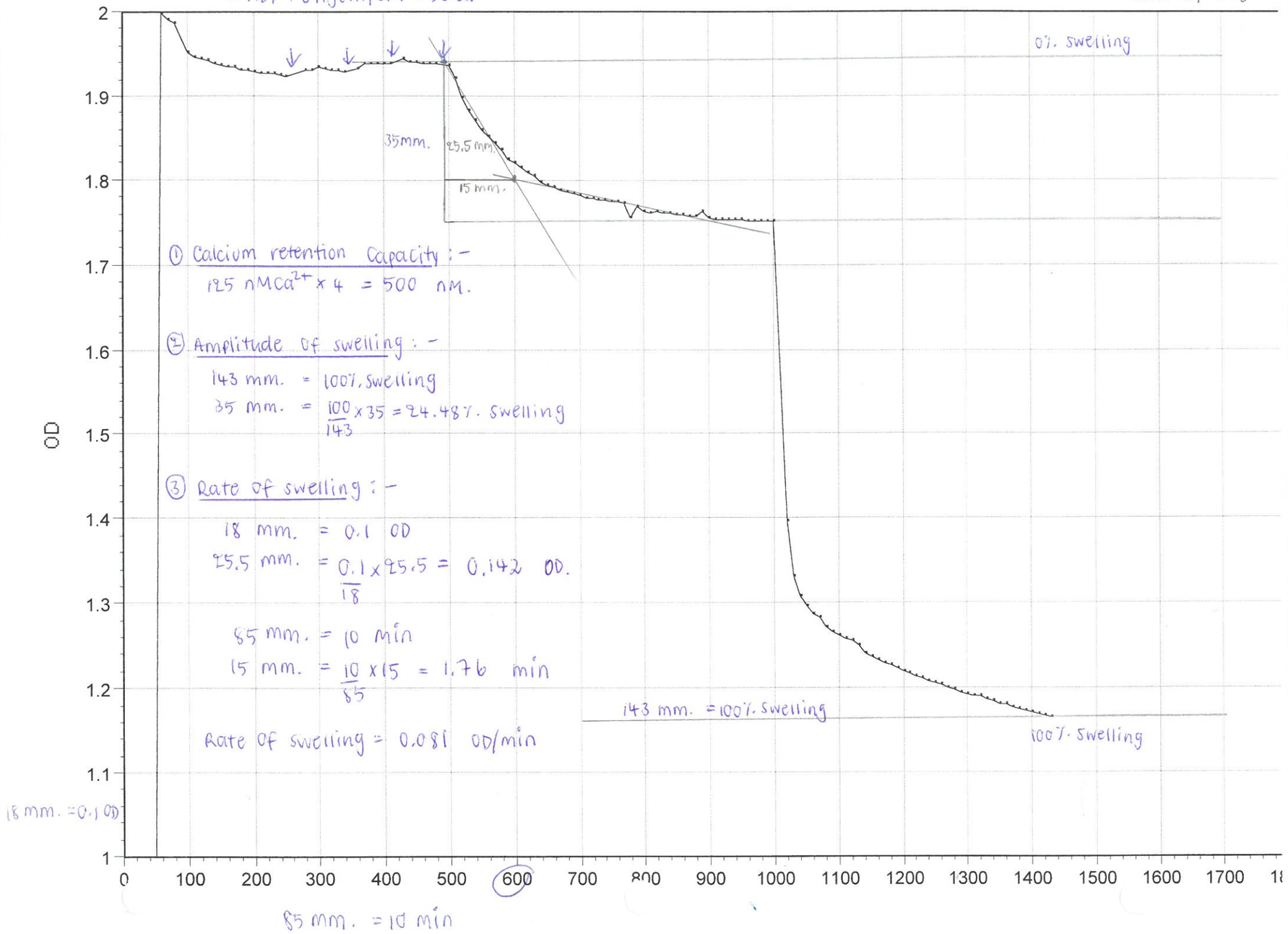
SSM

FGF2 + PKCε inhibitor

• ADP + Oligomycin + 50 Ca^{2+}

A2

Jan 16, 2008



① Calcium retention Capacity :-

125 nM Ca^{2+} × 4 = 500 nM.

② Amplitude of swelling :-

143 mm. = 100% swelling

35 mm. = $\frac{100}{143} \times 35 = 24.48\%$ swelling

③ Rate of swelling :-

18 mm. = 0.1 OD

25.5 mm. = $\frac{0.1}{18} \times 25.5 = 0.142$ OD.

85 mm. = 10 min

15 mm. = $\frac{10}{85} \times 15 = 1.76$ min

Rate of swelling = 0.081 OD/min

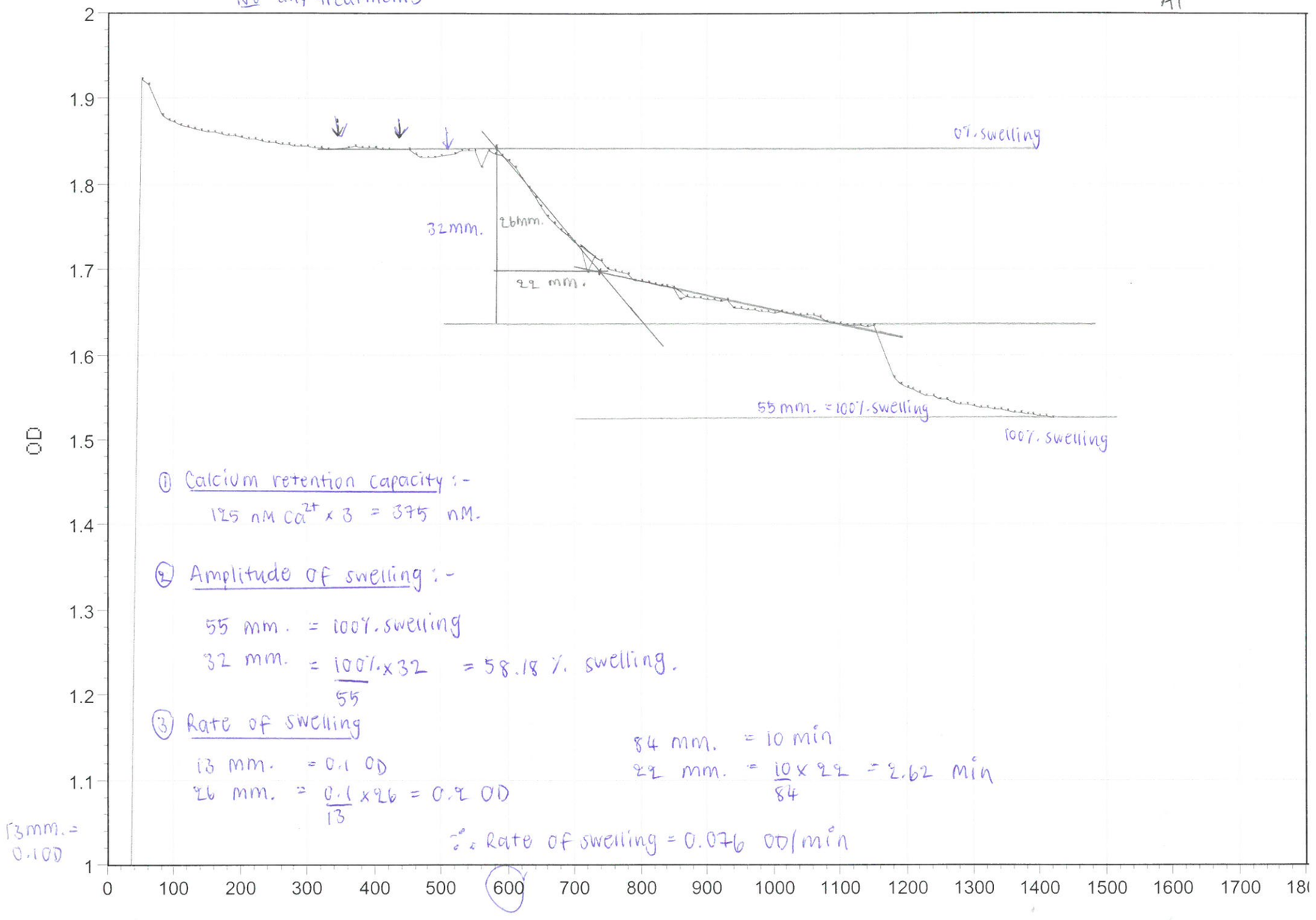
SSM

Control : ADP + Oligomycin.

Jan 18, 2008

A1

No any treatments



① Calcium retention capacity :-

125 nM Ca^{2+} × 3 = 375 nM.

② Amplitude of swelling :-

55 mm. = 100% swelling

32 mm. = $\frac{100\% \times 32}{55}$ = 58.18 % swelling.

③ Rate of swelling

13 mm. = 0.1 OD

26 mm. = $\frac{0.1}{13} \times 26$ = 0.2 OD

84 mm. = 10 min

22 mm. = $\frac{10}{84} \times 22$ = 2.62 min

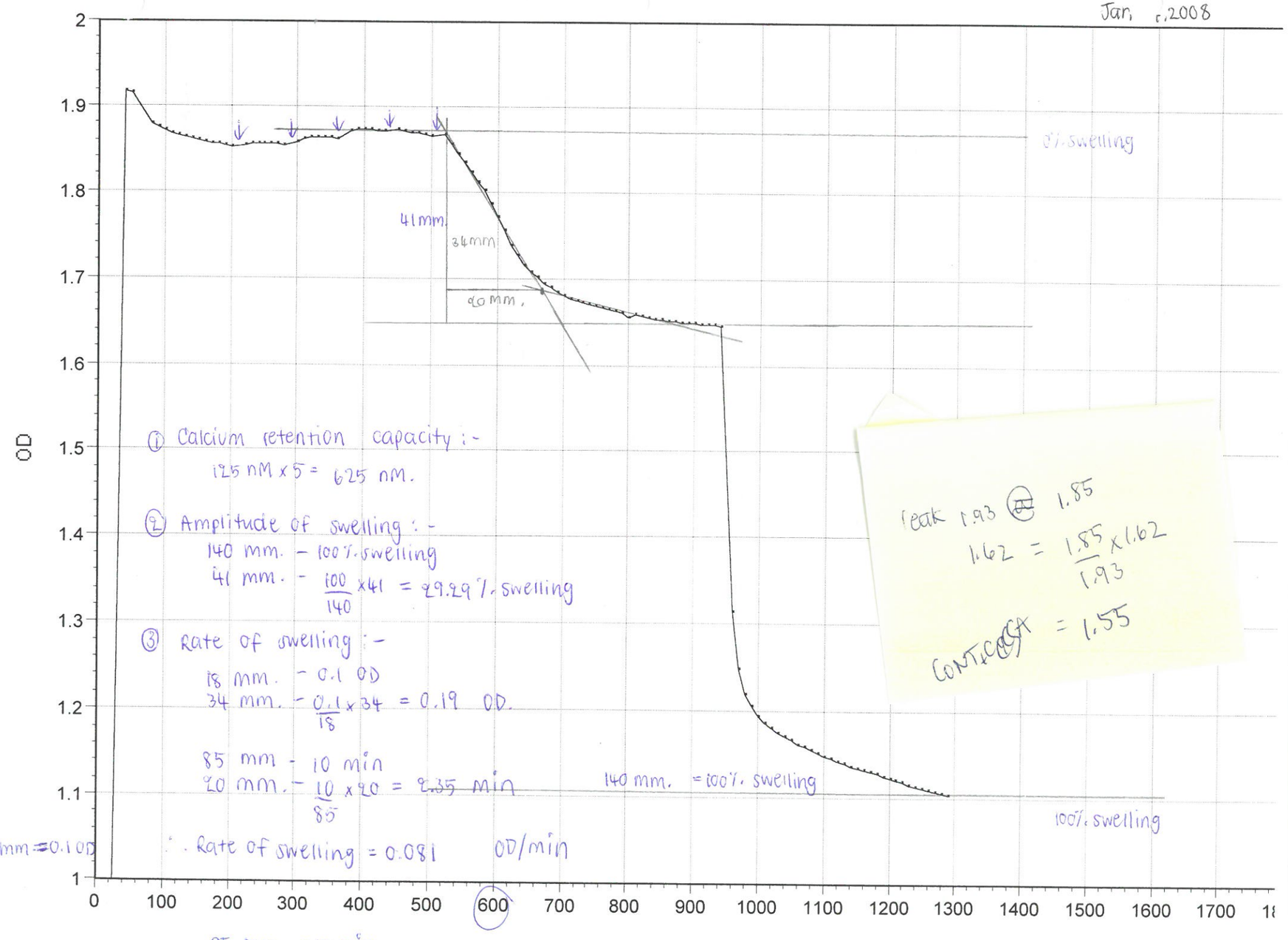
∴ Rate of swelling = 0.076 OD/min

SSM

ADP + Oligomycin (x5)

A1 (RHM)

Jan , 2008



① Calcium retention capacity :-

125 nM x 5 = 625 nM.

② Amplitude of swelling :-

140 mm. - 100% swelling

41 mm. - $\frac{100}{140} \times 41$ = 29.29 % swelling

③ Rate of swelling :-

18 mm. - 0.1 OD

34 mm. - $\frac{0.1}{18} \times 34$ = 0.19 OD.

85 mm - 10 min

20 mm. - $\frac{10}{85} \times 20$ = ~~2.35 min~~

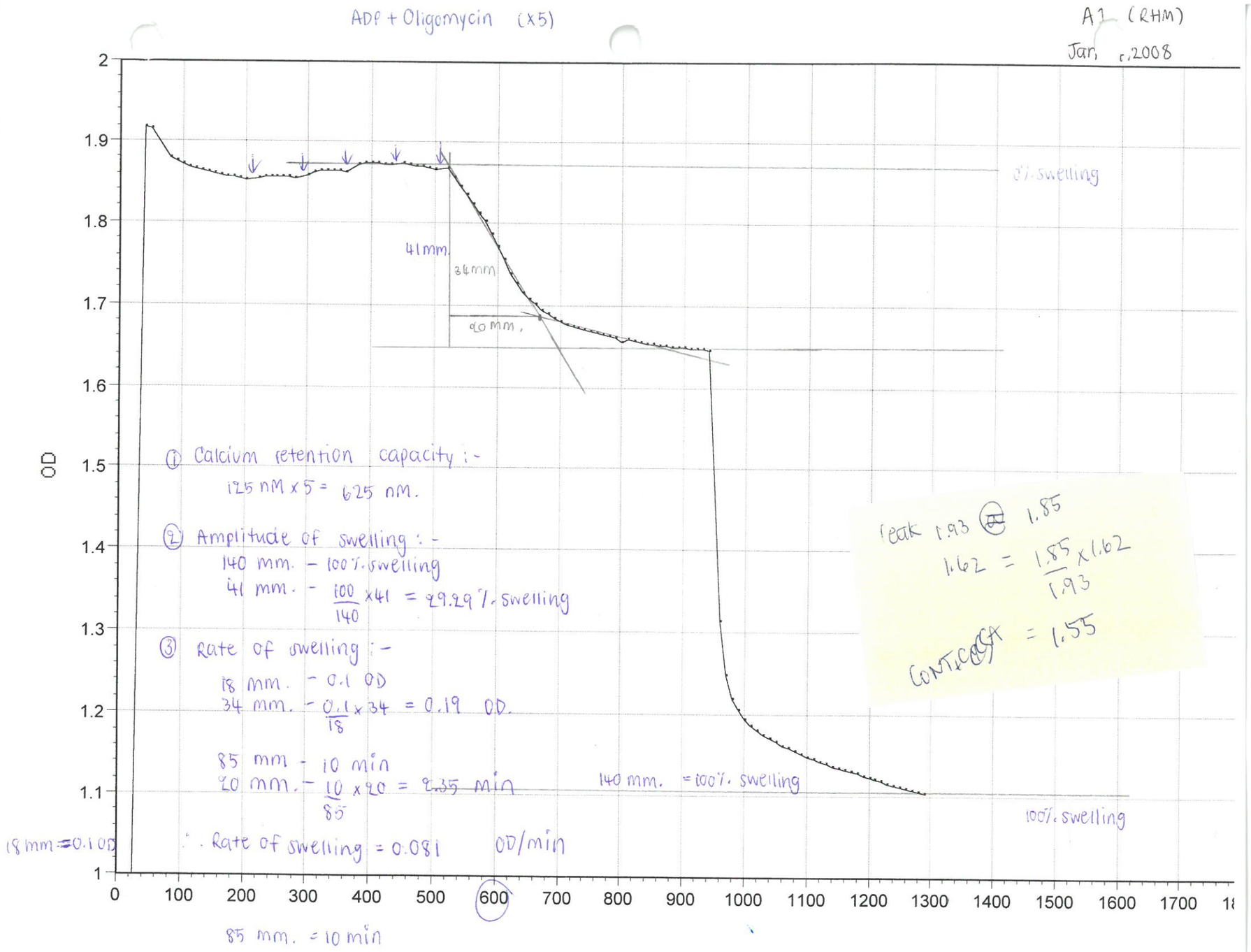
∴ Rate of swelling = 0.081 OD/min

Peak 1.93 ⓐ 1.85

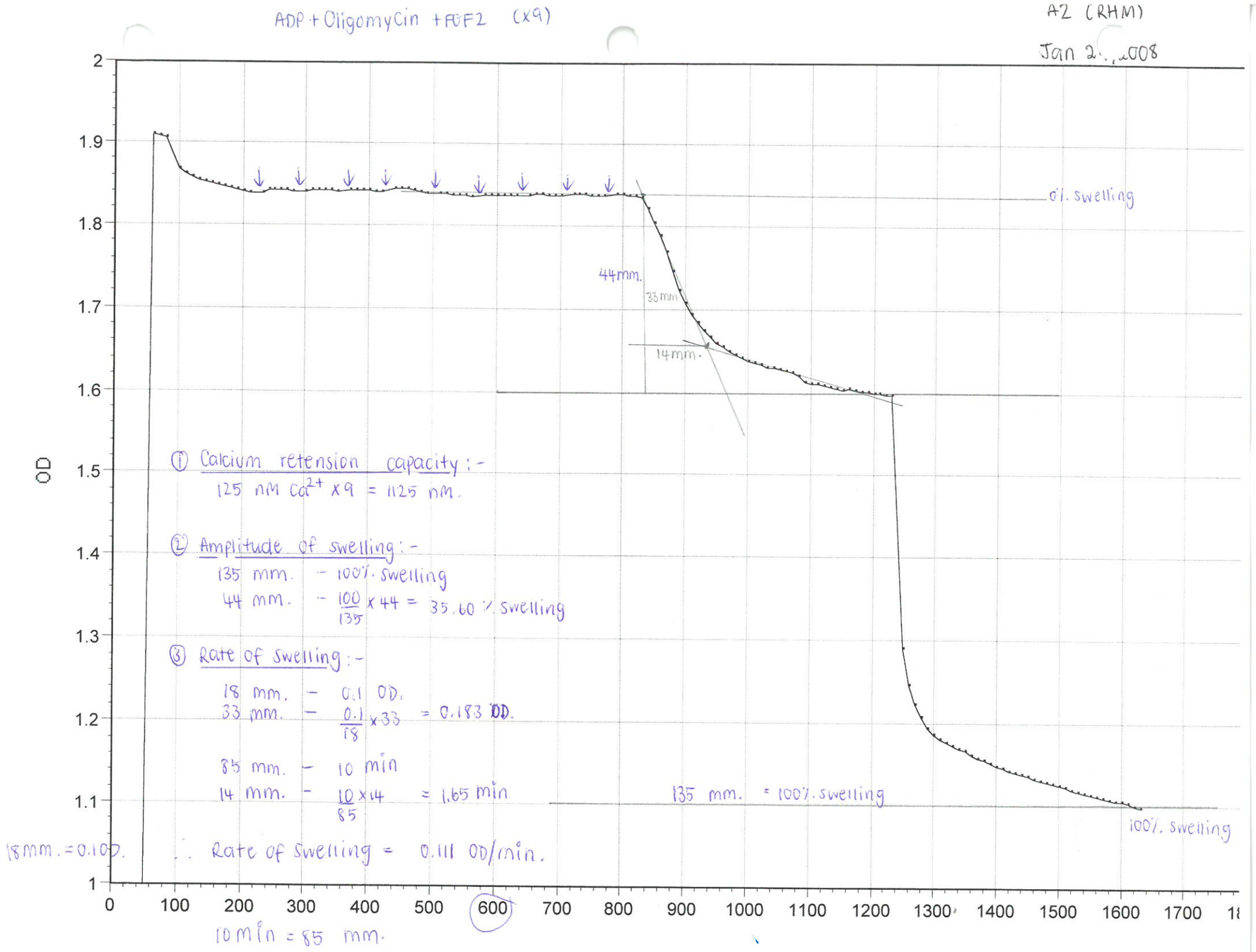
$1.62 = \frac{1.85}{1.93} \times 1.62$

CONTROL = 1.55

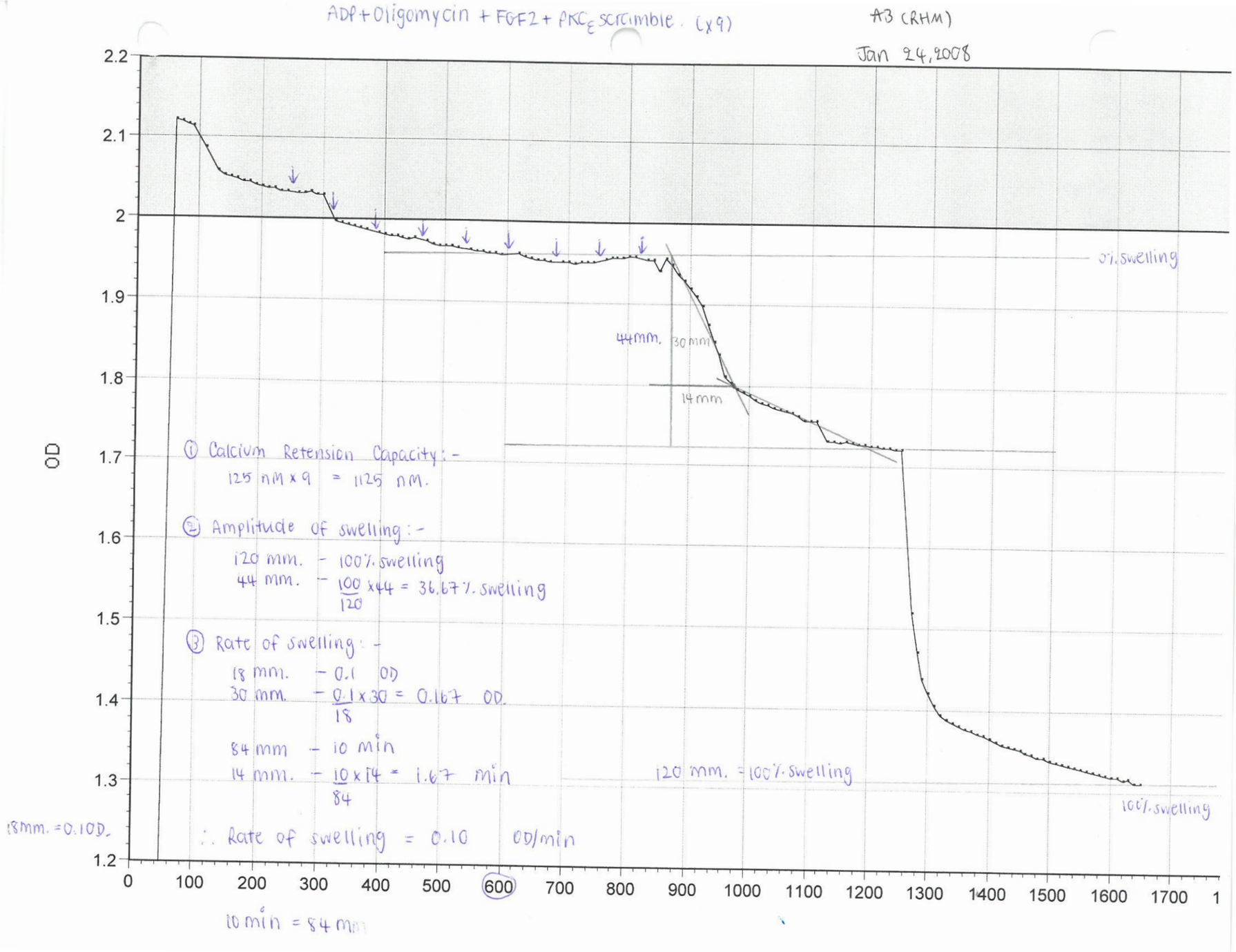
SSM


ADP + Oligomycin (x5)
A1 (RHM)
Jan, 2008
OD
0% swelling
41mm.
34mm
20 mm.
① Calcium retention capacity :-
125 nM x 5 = 625 nM.
② Amplitude of swelling :-
140 mm. - 100% swelling
41 mm. - 100/140 x41 = 29.29 % swelling
③ Rate of swelling :-
18 mm. - 0.1 OD
34 mm. - 0.1 x 34 / 18 = 0.19 OD.
85 mm - 10 min
20 mm. - 10 x 20 / 85 = 2.35 min
18 mm = 0.1 OD
∴ Rate of swelling = 0.081 OD/min
140 mm. = 100% swelling
100% swelling
85 mm. = 10 min
Peak 1.93 ⓐ 1.85
1.62 = 1.85 x 1.62 / 1.93
CONTROL = 1.55

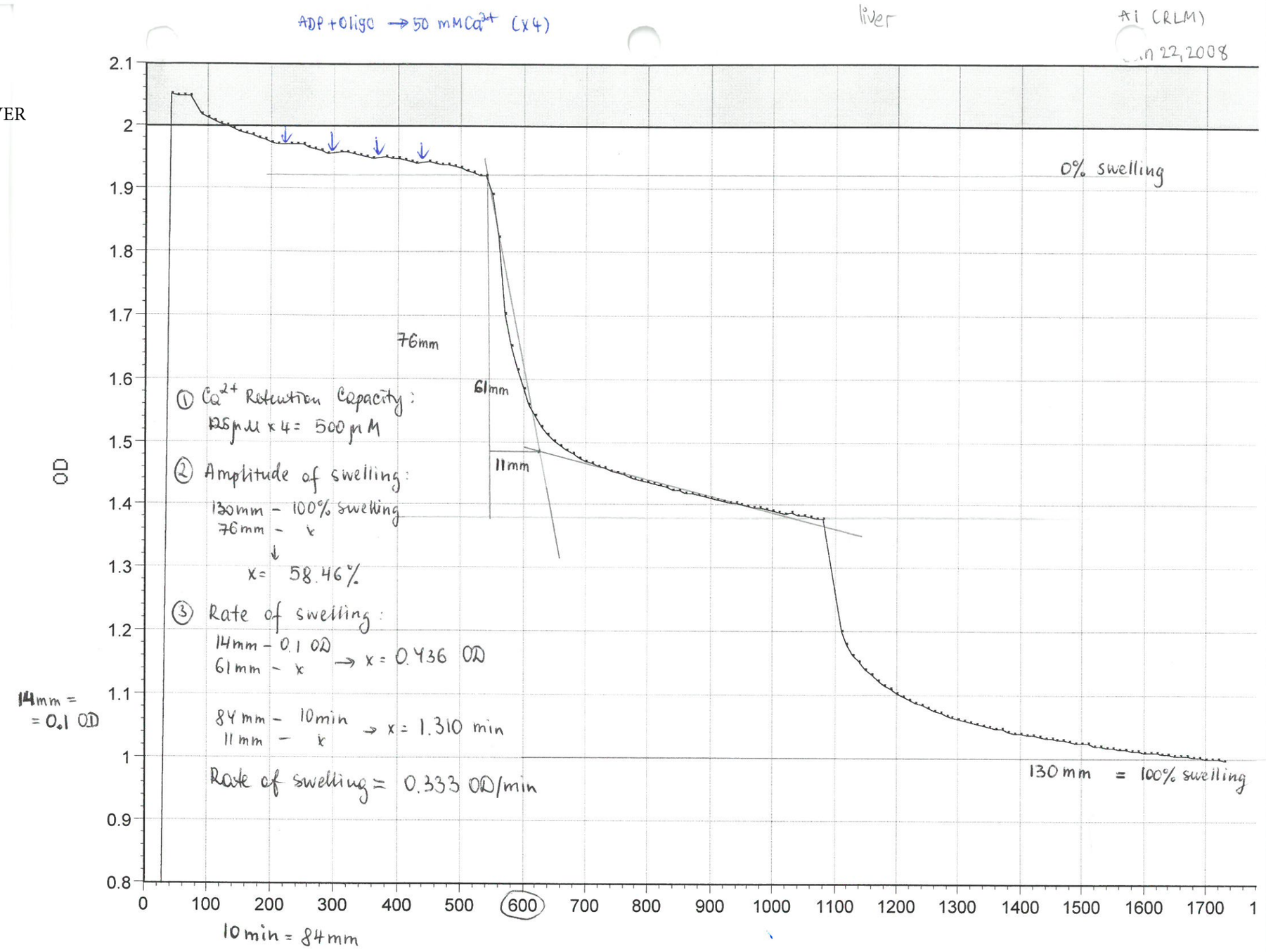
SSM


ADP + Oligomycin + FCCP (x9)
AZ (RHM)
Jan 2.., 2008
OD
0% swelling
44mm.
33 mm
14mm.
135 mm. = 100% swelling
100% swelling
18mm. = 0.1 OD.
10 min = 85 mm.
① Calcium retension capacity :-
125 nM Ca2+ x 9 = 1125 nM.
② Amplitude of swelling :-
135 mm. - 100% swelling
44 mm. - 100/135 x 44 = 35.60 % swelling
③ Rate of swelling :-
18 mm. - 0.1 OD.
33 mm. - 0.1/18 x 33 = 0.183 OD.
85 mm. - 10 min
14 mm. - 10/85 x 14 = 1.65 min
∴ Rate of Swelling = 0.111 OD/min.

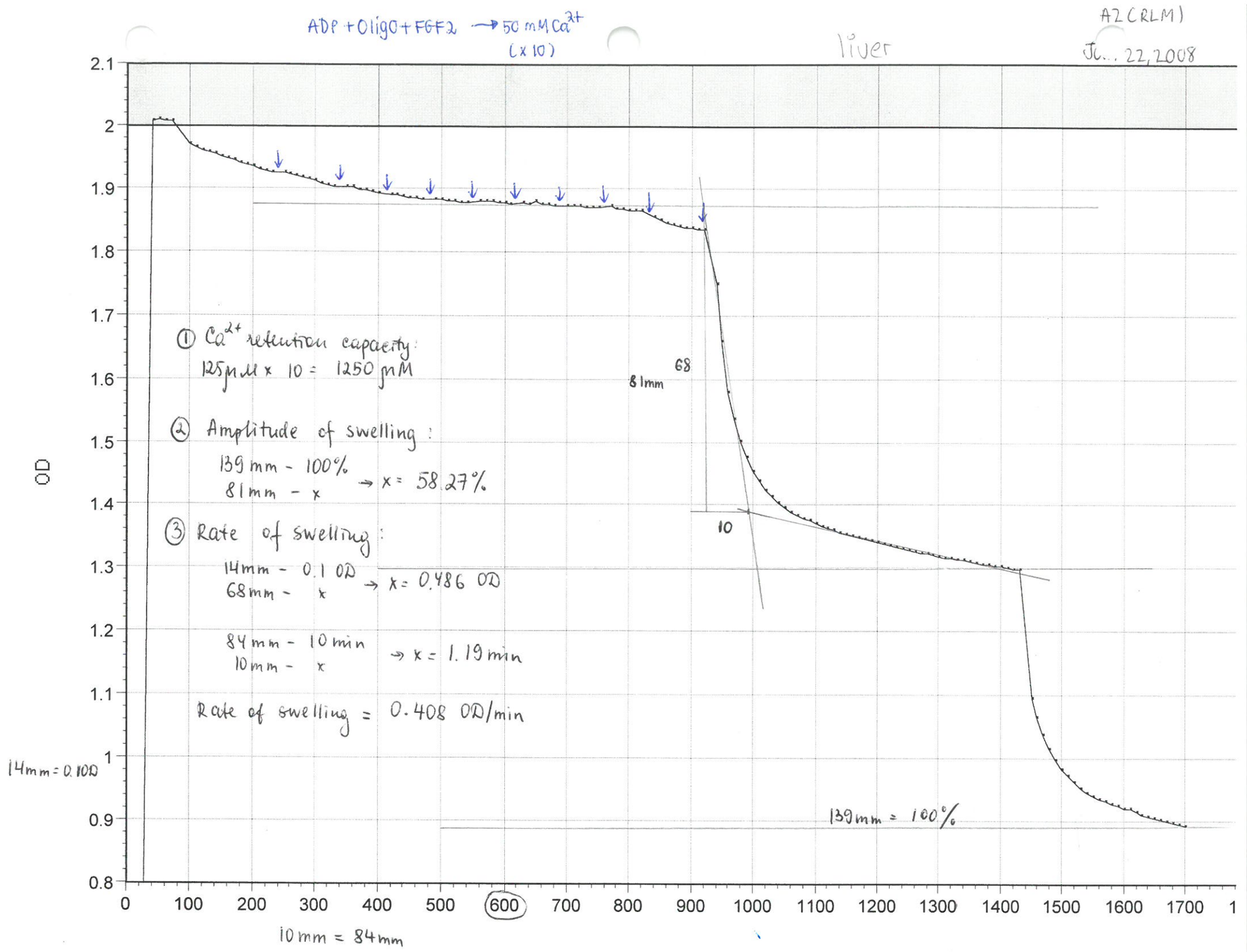
SSM


ADP + Oligomycin + FGF2 + PKCε scramble. (x9)
A3 (RHM)
Jan 24, 2008
OD
0% swelling
44mm.
30 mm
14 mm
① Calcium Retension Capacity:-
125 nM x 9 = 1125 nM.
② Amplitude of swelling:-
120 mm. - 100% swelling
44 mm. - 100/120 x44 = 36.67% swelling
③ Rate of swelling:-
18 mm. - 0.1 OD
30 mm. - 0.1/18 x 30 = 0.167 OD.
84 mm - 10 min
14 mm. - 10/84 x 14 = 1.67 min
∴ Rate of swelling = 0.10 OD/min
120 mm. = 100% swelling
100% swelling
18mm. = 0.10D.
10 min = 84 mm

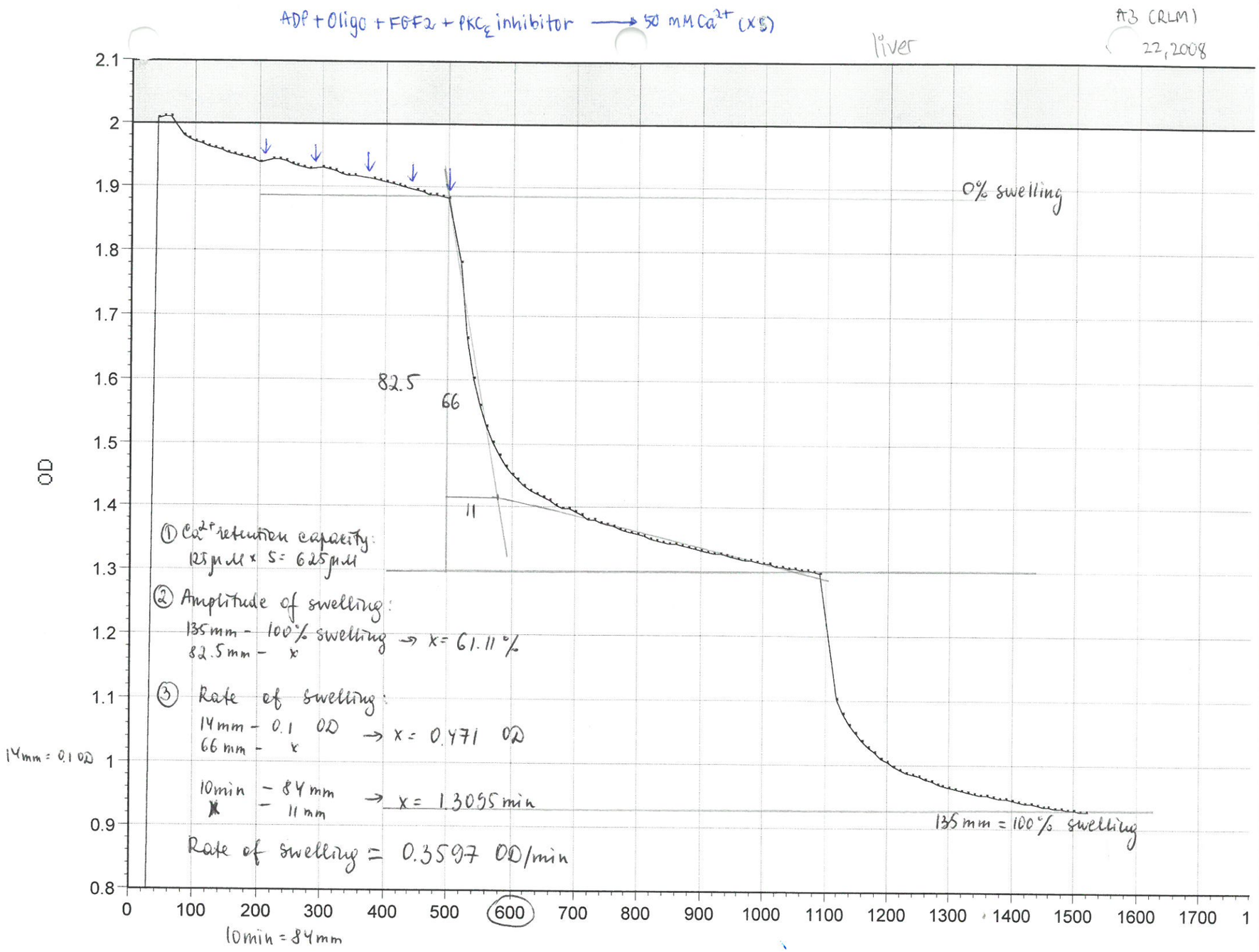
LIVER


ADP + Oligo → 50 mM Ca^{2+} (x4)
liver
Ai (RLM)
Jun 22, 2008
0% swelling
76mm
61mm
11mm
① Ca^{2+} Retention Capacity:
125 μM x 4 = 500 μM
② Amplitude of swelling:
130mm - 100% swelling
76mm - x
x = 58.46%
③ Rate of swelling:
14mm - 0.1 OD
61mm - x → x = 0.436 OD
84mm - 10min
11mm - x → x = 1.310 min
Rate of swelling = 0.333 OD/min
14mm = 0.1 OD
130 mm = 100% swelling
10 min = 84mm
OD
2.1
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700

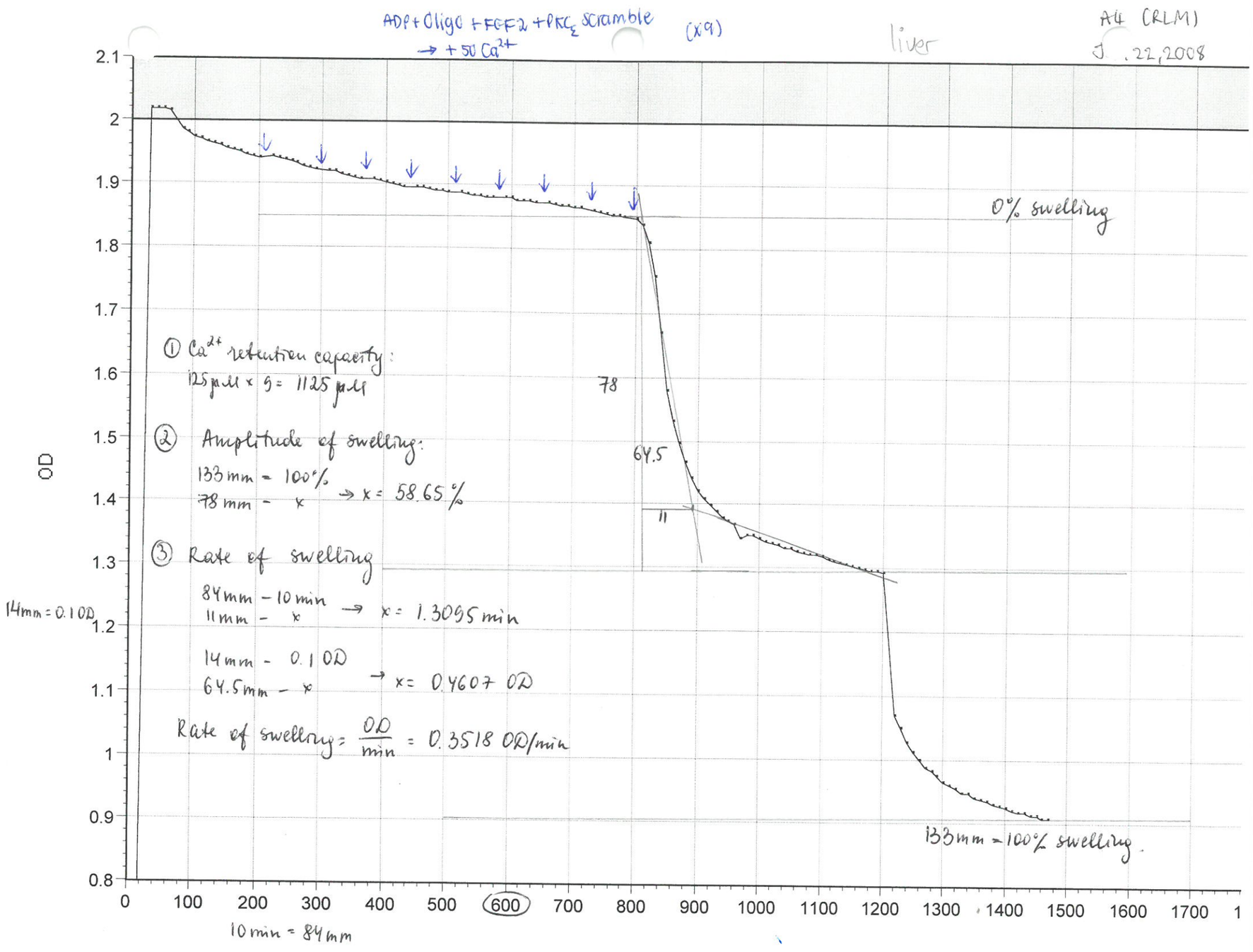
LIVER


ADP + Oligo + FGF2 → 50 mM Ca^{2+} (x 10)
liver
A2 (RLM)
Jl.. 22, 2008
OD
2.1
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0 100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600 1700 1
10 mm = 84 mm
14mm = 0.10D
① Ca^{2+} retention capacity:
125 μM x 10 = 1250 μM
② Amplitude of swelling:
139 mm - 100%
81 mm - x → x = 58.27%
③ Rate of swelling:
14mm - 0.1 OD
68mm - x → x = 0.486 OD
84 mm - 10 min
10 mm - x → x = 1.19 min
Rate of swelling = 0.408 OD/min
81mm
68
10
139mm = 100%

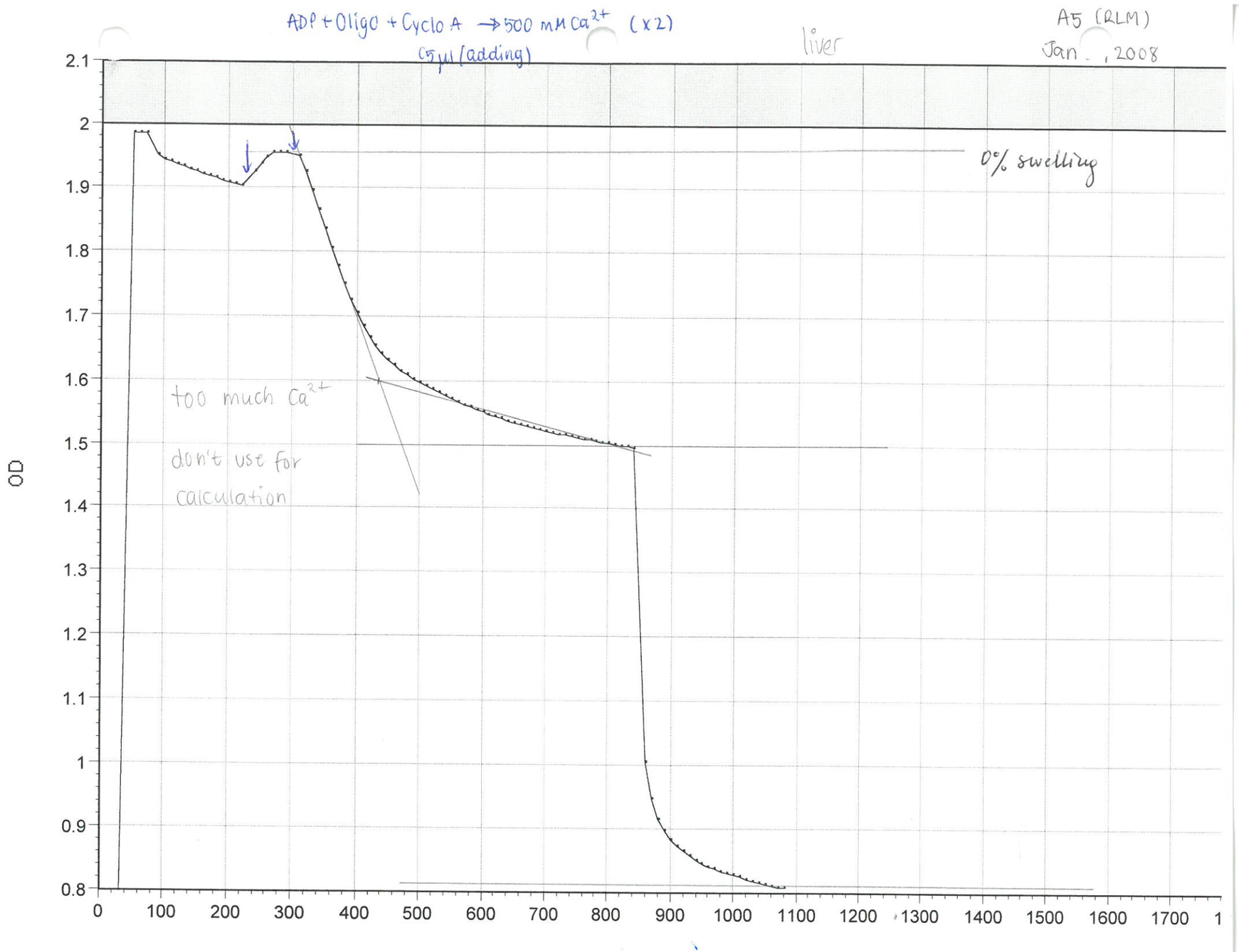
LIVER


ADP + Oligo + FCF2 + PKCε inhibitor → 50 mM Ca2+ (x5)
liver
AB (CRLM)
22, 2008
OD
0% swelling
82.5
66
11
① Ca2+ retention capacity:
125 μM x 5 = 625 μM
② Amplitude of swelling:
135 mm – 100% swelling → x = 61.11 %
82.5 mm – x
③ Rate of swelling:
14 mm – 0.1 OD → x = 0.471 OD
66 mm – x
10 min – 84 mm → x = 1.3095 min
x – 11 mm
Rate of swelling = 0.3597 OD/min
135 mm = 100% swelling
14mm = 0.1 OD
10min = 84mm

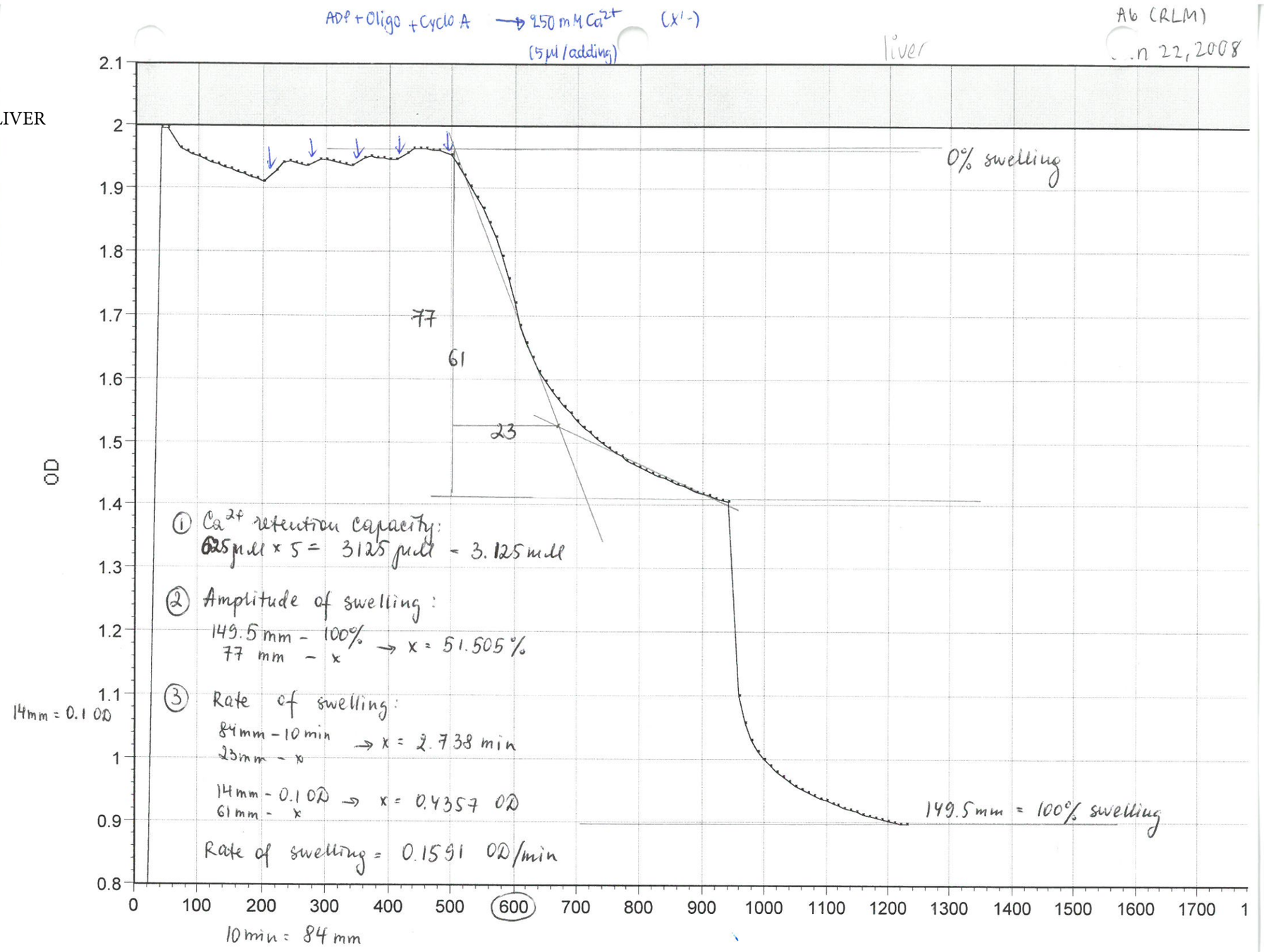
LIVER


ADP + Oligo + FGF2 + PKCε scramble
→ + 50 Ca2+
(x9)
liver
A4 (RLM)
J. 22,2008
OD
0% swelling
① Ca2+ retention capacity:
125 µM x 9 = 1125 µM
78
② Amplitude of swelling:
133 mm = 100%
78 mm = x → x = 58.65 %
64.5
11
③ Rate of swelling
84 mm – 10 min
11 mm – x → x = 1.3095 min
14 mm = 0.1 OD
14 mm – 0.1 OD
64.5 mm – x → x = 0.4607 OD
Rate of swelling = OD/min = 0.3518 OD/min
133 mm = 100% swelling.
10 min = 84 mm

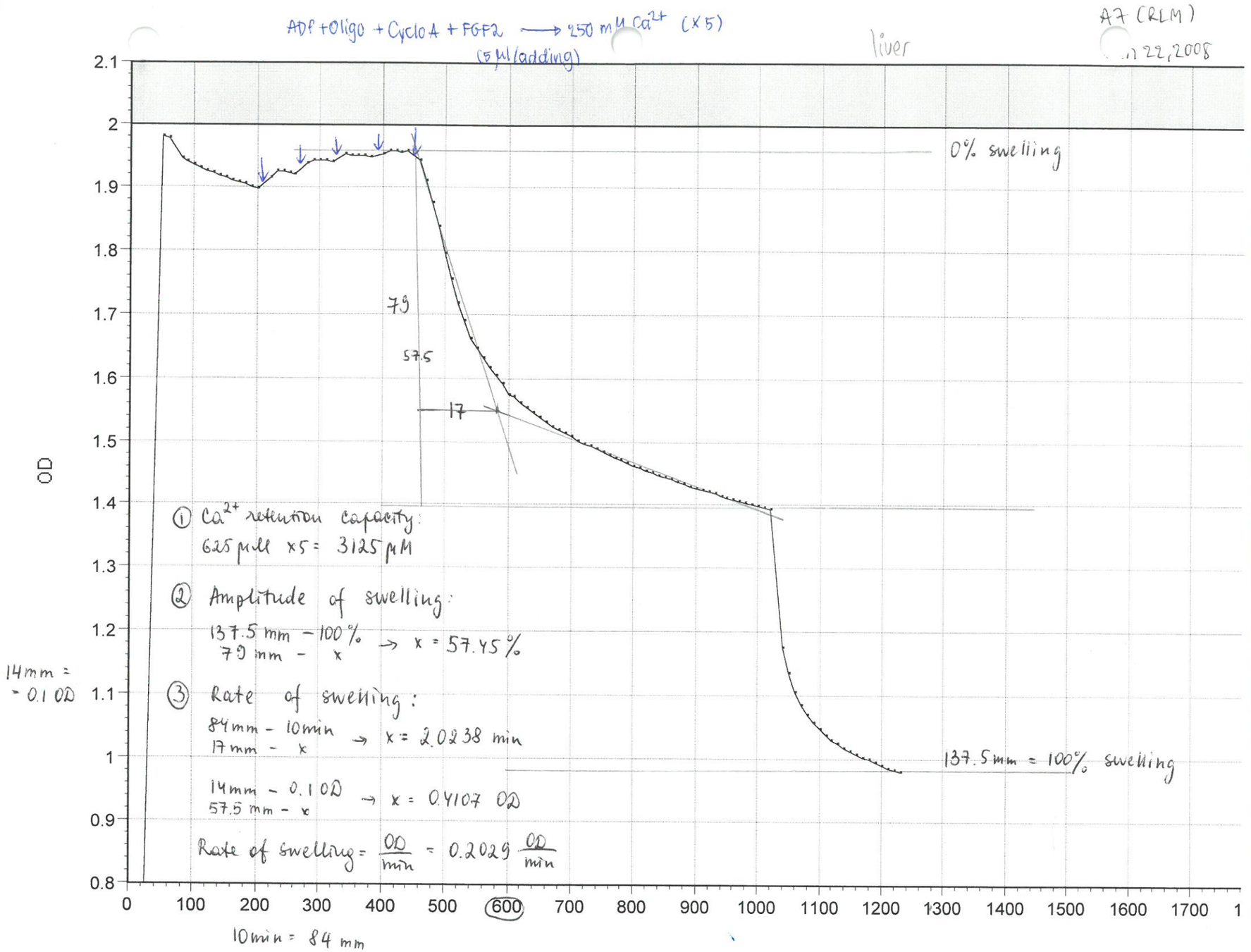
LIVER


ADP + Oligo + Cyclo A → 500 mM Ca^{2+} (x2)
5 μl (adding)
liver
A5 (RLM)
Jan _, 2008
0% swelling
too much Ca^{2+}
don't use for calculation
OD
2.1
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700

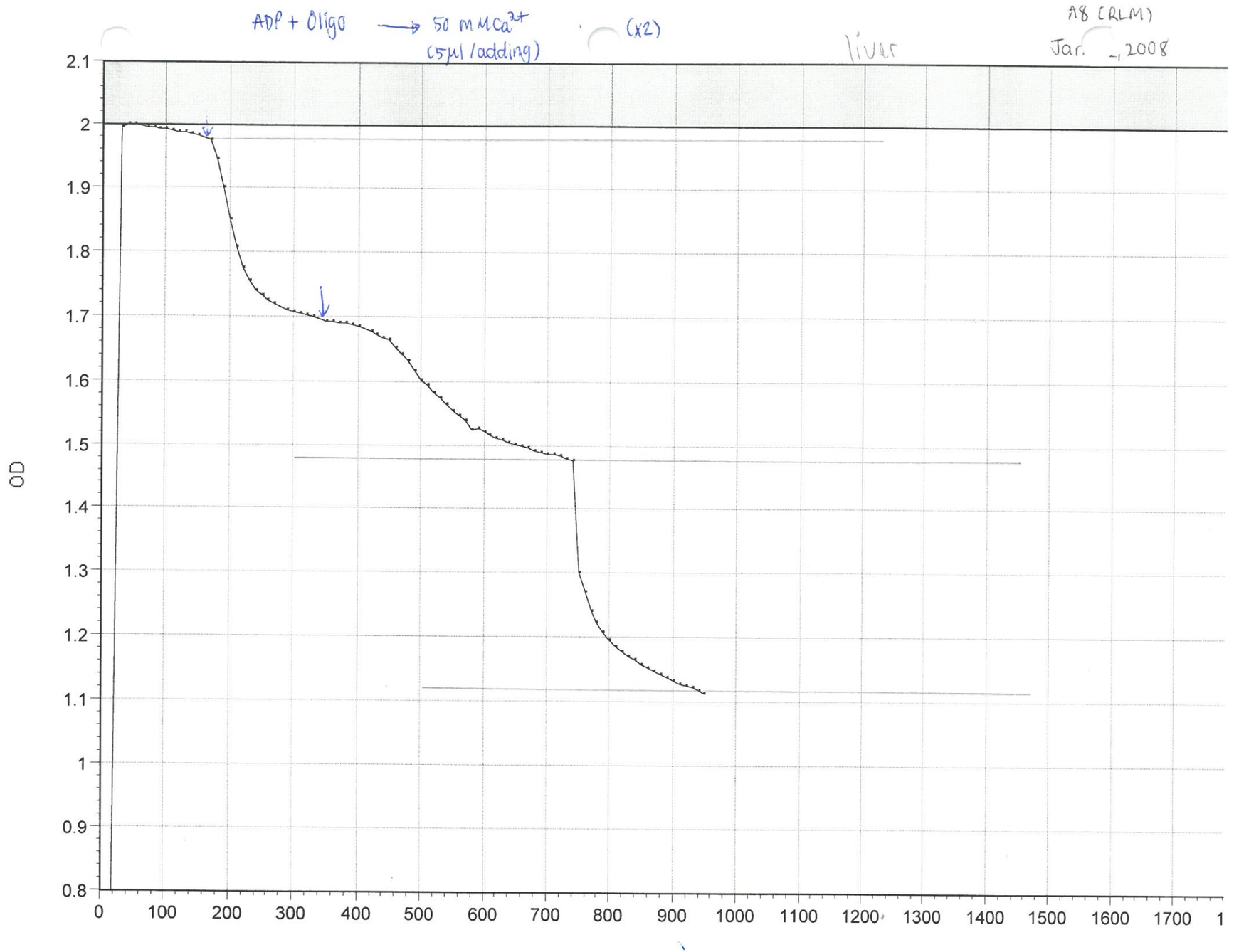
LIVER


ADP + Oligo + Cyclo A → 250 mM Ca^{2+} (X'-)
(5 µl / adding)
liver
A6 (RLM)
..n 22, 2008
OD
0% swelling
77
61
23
① Ca^{2+} retention capacity:
625 µM x 5 = 3125 µM = 3.125 mM
② Amplitude of swelling:
149.5 mm - 100%
77 mm - x
→ x = 51.505 %
③ Rate of swelling:
84 mm - 10 min
23 mm - x
→ x = 2.738 min
14 mm - 0.1 OD
61 mm - x
→ x = 0.4357 OD
Rate of swelling = 0.1591 OD/min
14mm = 0.1 OD
149.5 mm = 100% swelling
10 min = 84 mm

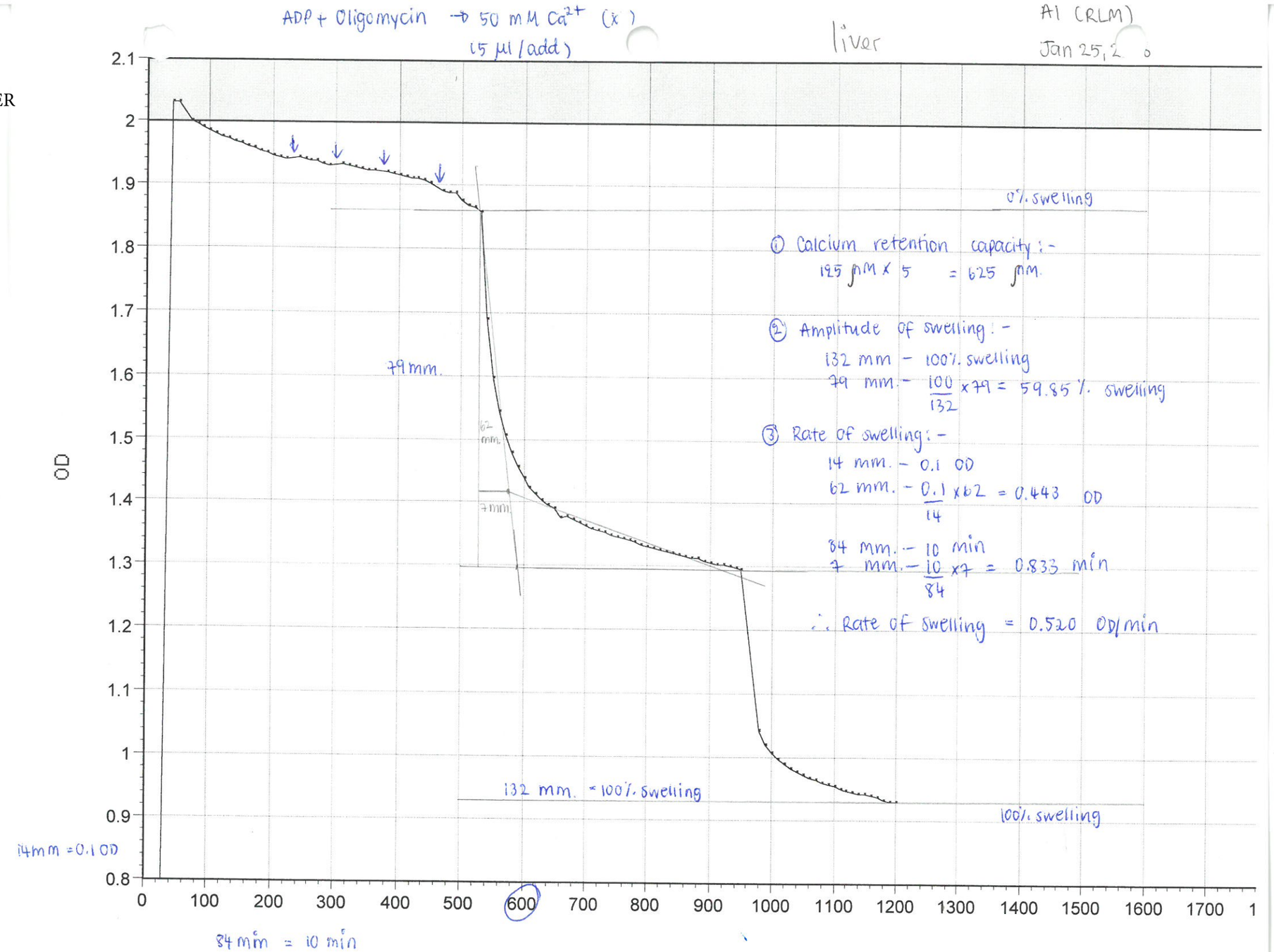
LIVER


ADP + Oligo + Cyclo A + FGF2 → 250 mM Ca^{2+} (X5)
(5 µl/adding)
liver
A7 (RLM)
22, 2008
0% swelling
79
57.5
17
OD
2.1
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
14mm = 0.1 OD
① Ca^{2+} retention capacity:
625 µM x5 = 3125 µM
② Amplitude of swelling:
137.5 mm – 100 % → x = 57.45 %
79 mm – x
③ Rate of swelling:
84 mm – 10 min → x = 2.0238 min
17 mm – x
14 mm – 0.1 OD → x = 0.4107 OD
57.5 mm – x
Rate of swelling = OD/min = 0.2029 OD/min
137.5 mm = 100% swelling
0 100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600 1700 1
10 min = 84 mm

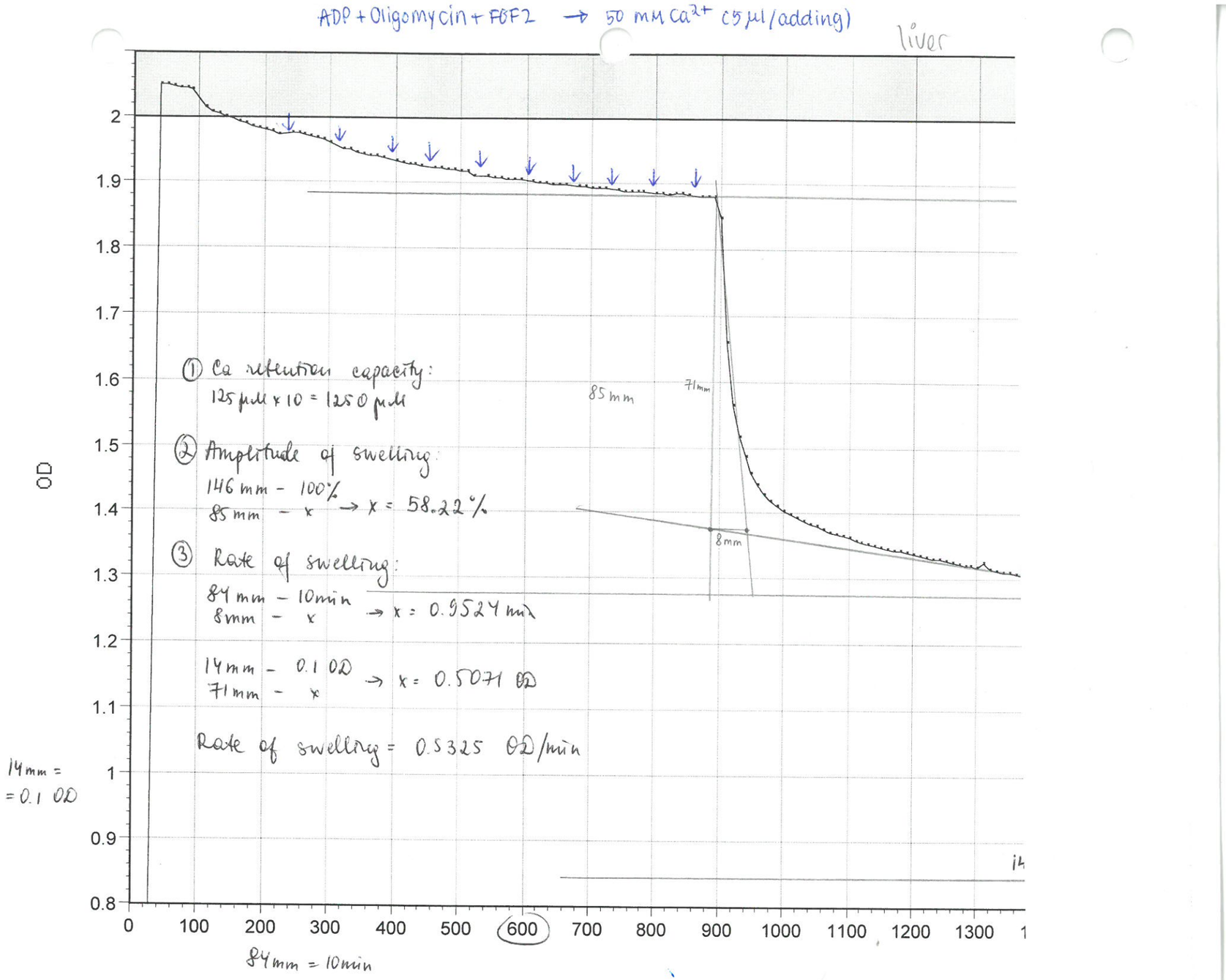
LIVER


ADP + Oligo → 50 mM Ca^{2+} (5μl / adding)
(x2)
liver
A8 (RLM)
Jan. -, 2008
OD
2.1
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700

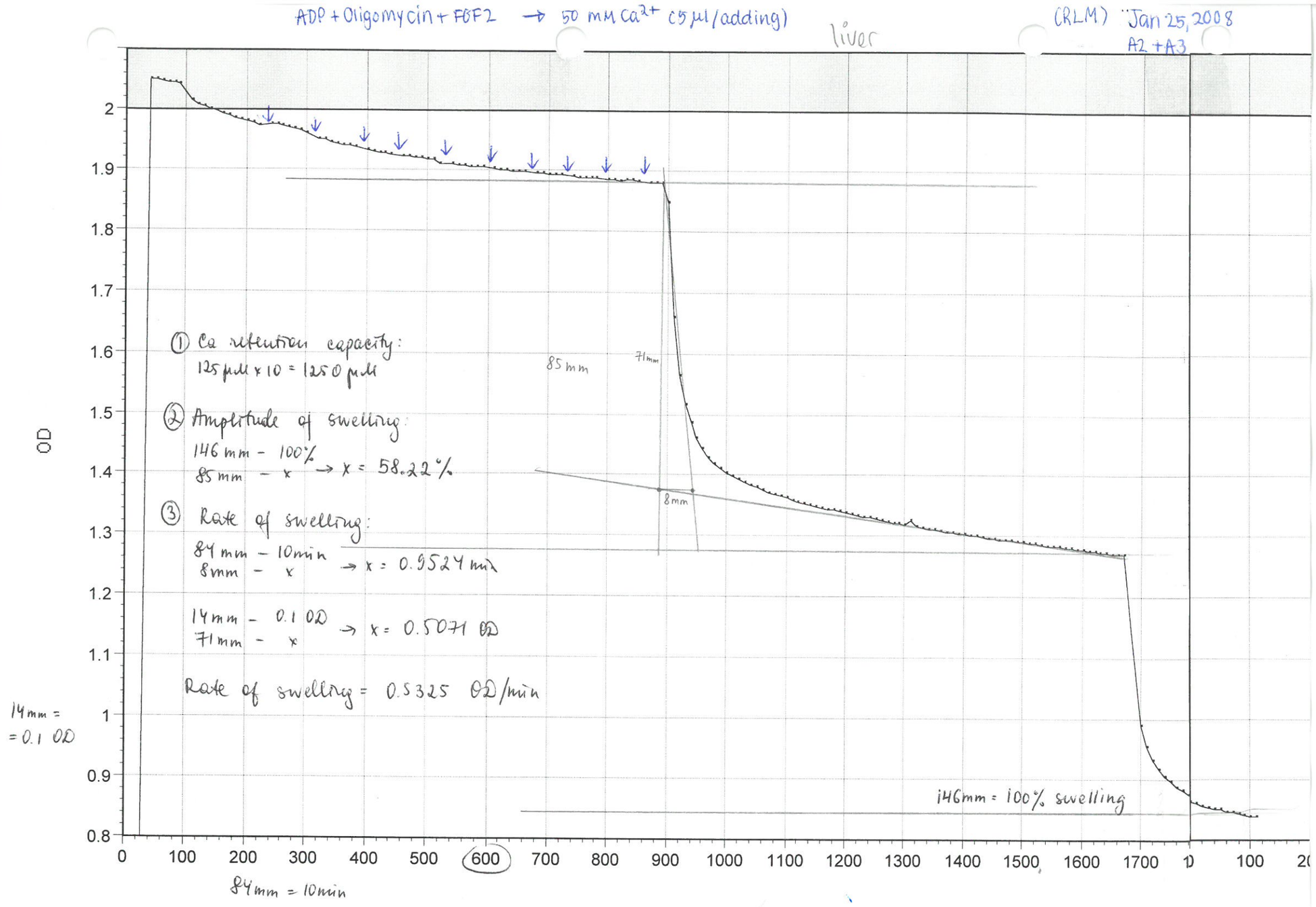
LIVER


ADP + Oligomycin → 50 mM Ca^{2+} (x)
15 µl / add)
liver
A1 (RLM)
Jan 25, 2
OD
79 mm.
62 mm.
7 mm.
0% swelling
100% swelling
132 mm. = 100% swelling
14mm = 0.1 OD
84 min = 10 min
① Calcium retention capacity :-
125 nM x 5 = 625 nM.
② Amplitude of swelling :-
132 mm - 100% swelling
79 mm. - 100/132 x 79 = 59.85 % swelling
③ Rate of swelling :-
14 mm. - 0.1 OD
62 mm. - 0.1/14 x 62 = 0.443 OD
84 mm. - 10 min
7 mm. - 10/84 x 7 = 0.833 min
∴ Rate of swelling = 0.520 OD/min

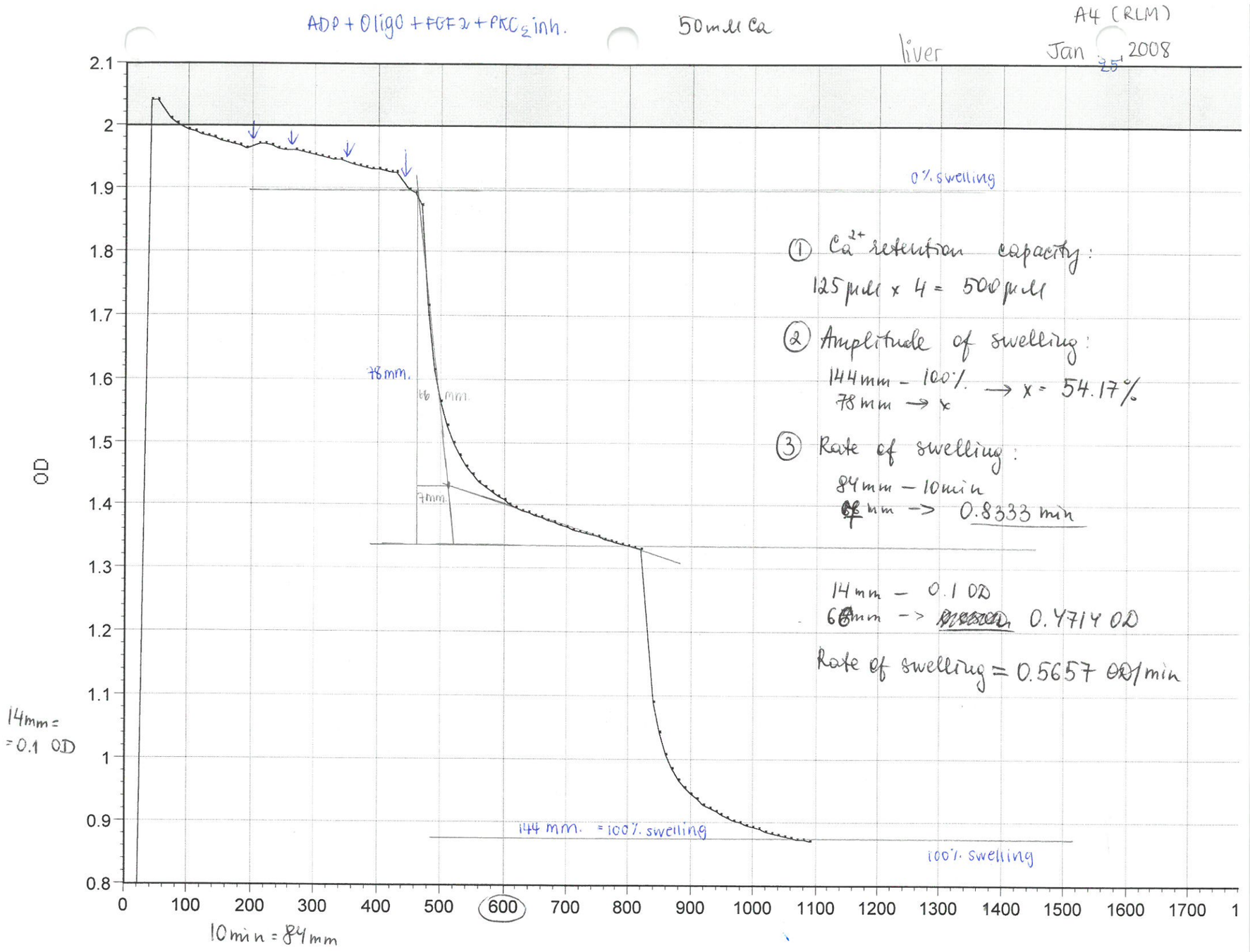
LIVER


ADP + Oligomycin + FCF2 → 50 mM Ca^{2+} (5 µl/adding)
liver
OD
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
① Ca retention capacity:
125 µM x 10 = 1250 µM
② Amplitude of swelling:
146 mm - 100%
85 mm - x → x = 58.22 %
③ Rate of swelling:
84 mm - 10min
8mm - x → x = 0.9524 min
14mm - 0.1 OD
71mm - x → x = 0.5071 OD
Rate of swelling = 0.5325 OD/min
85 mm
71mm
8mm
14mm = 0.1 OD
84mm = 10min

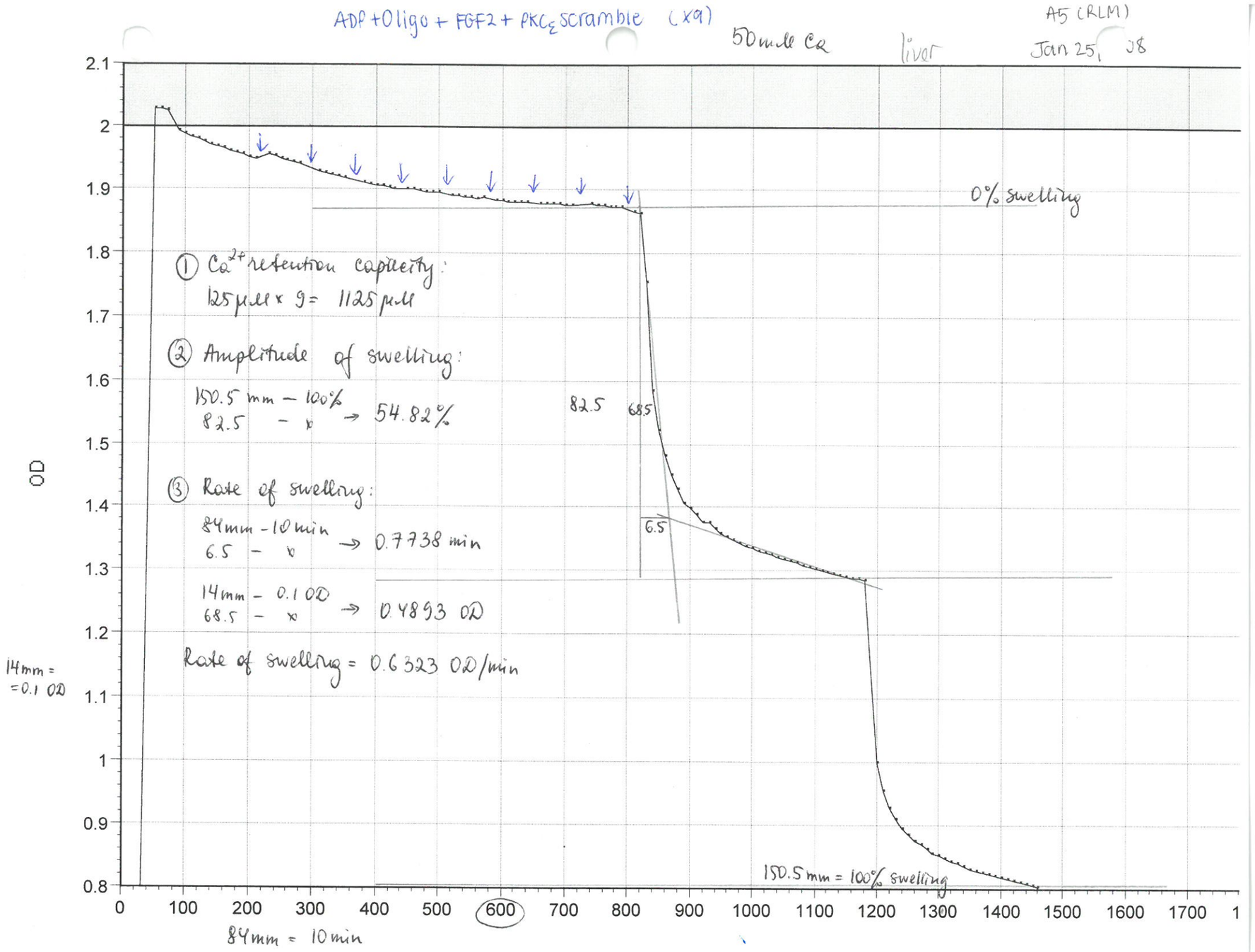
LIVER


ADP + Oligomycin + FBF2 → 50 mM Ca2+ (5 µl/adding)
liver
(RLM) Jan 25, 2008
A2 + A3
OD
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
① Ca retention capacity:
125 µM x 10 = 1250 µM
② Amplitude of swelling:
146 mm - 100%
85 mm - x → x = 58.22%
③ Rate of swelling:
84 mm - 10min
8mm - x → x = 0.9524 min
14mm - 0.1 OD
71mm - x → x = 0.5071 OD
Rate of swelling = 0.5325 OD/min
85 mm
71mm
8mm
14mm = 0.1 OD
146mm = 100% swelling
84mm = 10min

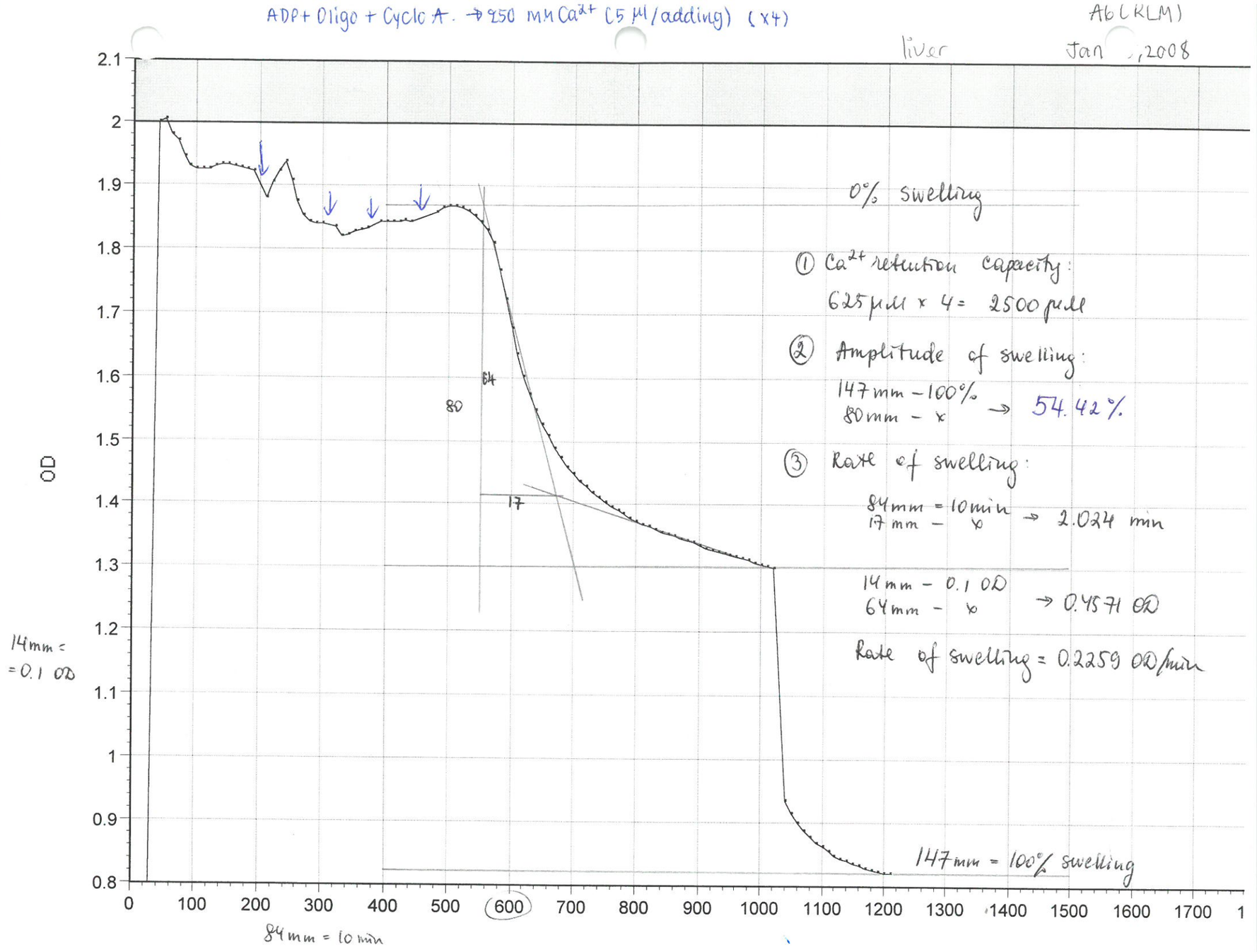
LIVER


ADP + Oligo + FCF2 + PKC$_\zeta$ inh.
50mM Ca
liver
A4 (RLM)
Jan 25 2008
OD
0% swelling
78mm.
66 mm.
7mm.
144 mm. = 100% swelling
100% swelling
10min = 84mm
14mm = = 0.1 OD
① Ca^{2+} retention capacity:
125 µM x 4 = 500 µM
② Amplitude of swelling:
144mm - 100% → x = 54.17%
78mm → x
③ Rate of swelling:
84mm - 10min
66mm → 0.8333 min
14mm - 0.1 OD
66mm → 0.4714 OD
Rate of swelling = 0.5657 OD/min

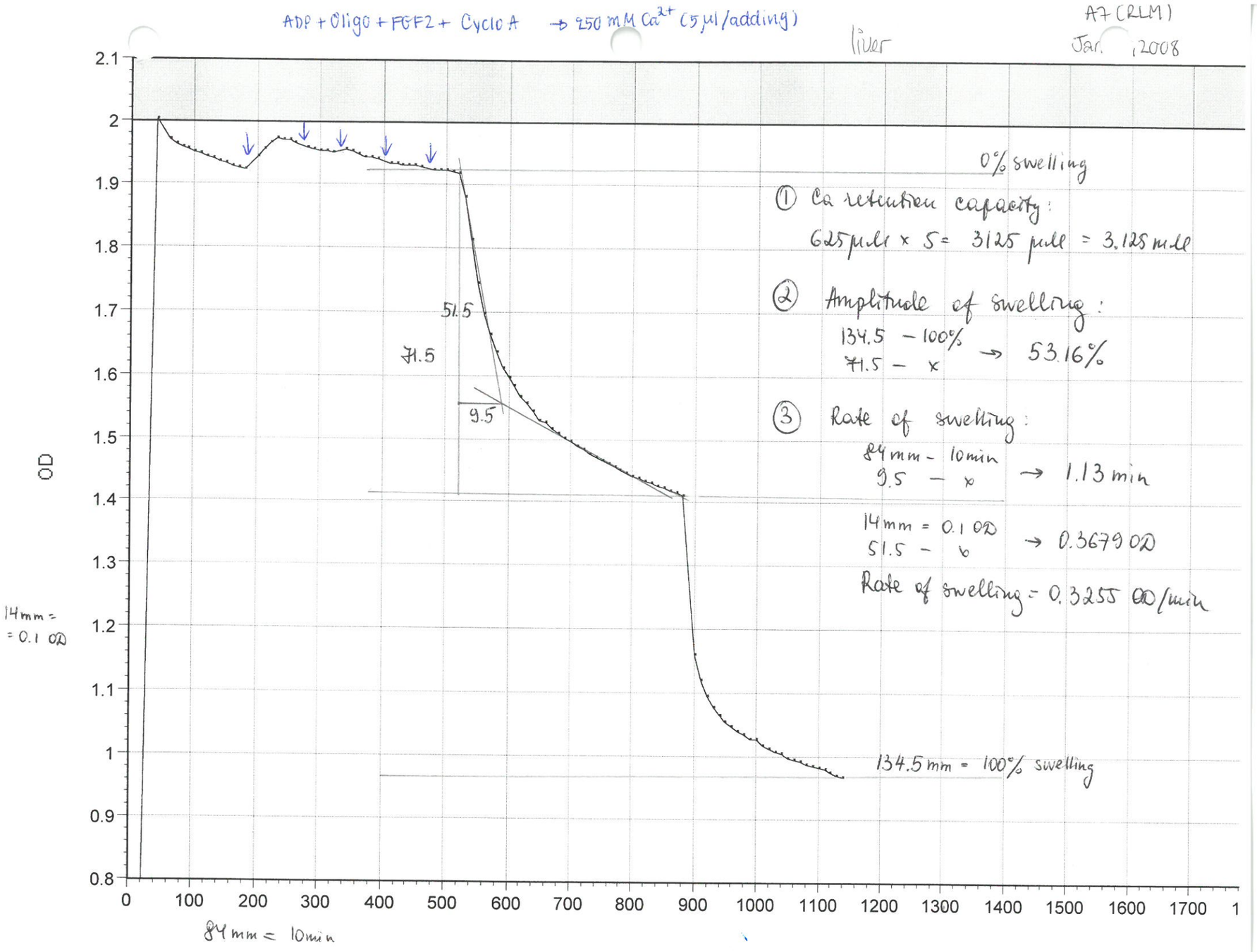
LIVER


ADP + Oligo + FGF2 + PKCε scramble (x9)
50mM Ca
liver
A5 (RLM)
Jan 25, 08
0% swelling
① Ca2+ retention capacity:
125 µl x 9 = 1125 µl
② Amplitude of swelling:
150.5 mm – 100%
82.5 – x → 54.82%
82.5
68.5
③ Rate of swelling:
84mm - 10 min
6.5 – x → 0.7738 min
14mm – 0.1 OD
68.5 – x → 0.4893 OD
Rate of swelling = 0.6323 OD/min
6.5
14mm = 0.1 OD
OD
150.5 mm = 100% swelling
84mm = 10 min
2.1
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700

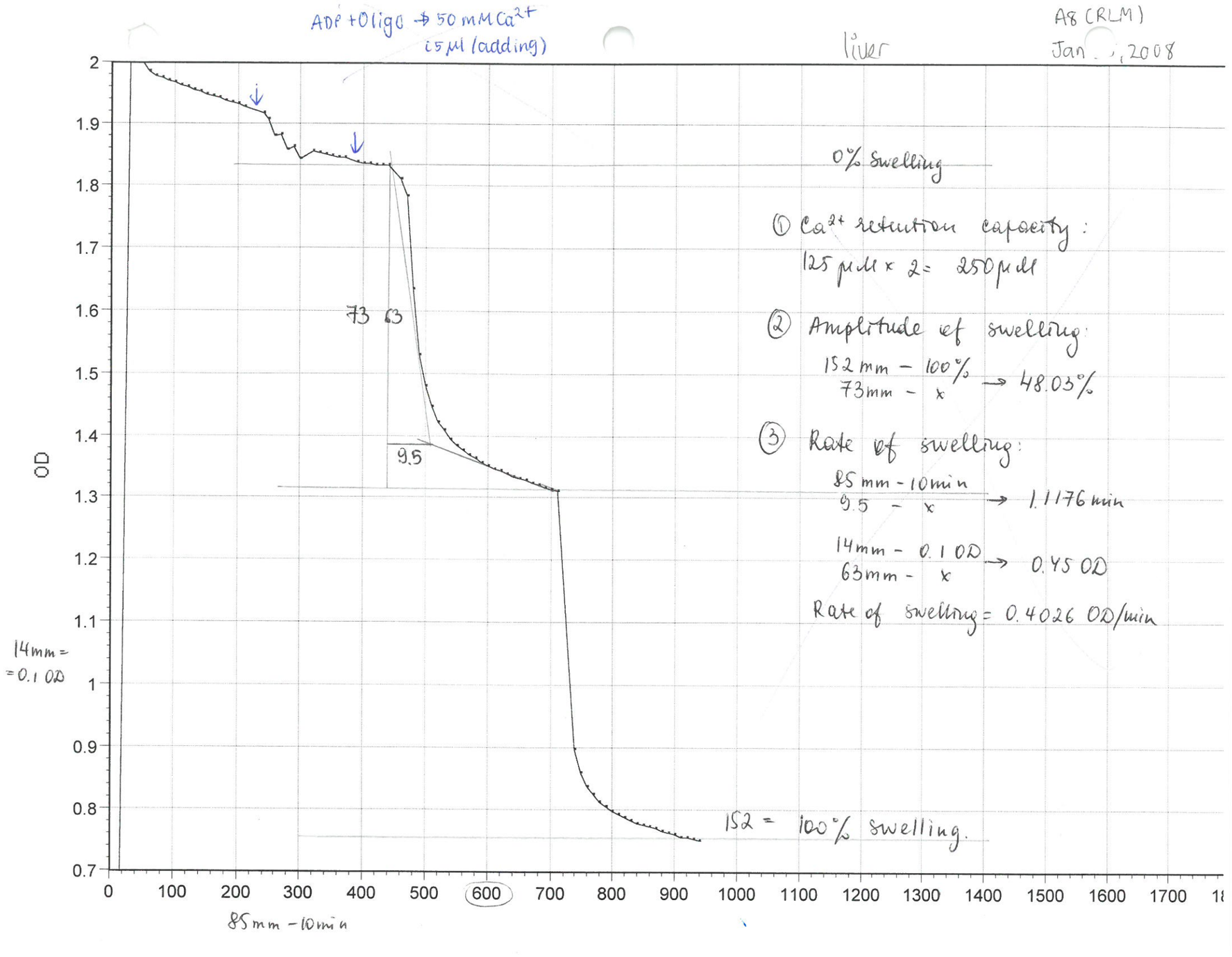
LIVER


ADP + Oligo + Cyclo A. → 250 mM Ca^{2+} (5 µl/adding) (x4)
liver
Jan ,2008
0% swelling
① Ca^{2+} retention capacity:
625 µM x 4 = 2500 µM
② Amplitude of swelling:
147 mm – 100%
80 mm – x → 54.42 %
③ Rate of swelling:
84 mm = 10 min
17 mm – x → 2.024 min
14 mm – 0.1 OD
64 mm – x → 0.4571 OD
Rate of swelling = 0.2259 OD/min
147 mm = 100% swelling
64
80
17
OD
14 mm = 0.1 OD
84 mm = 10 min

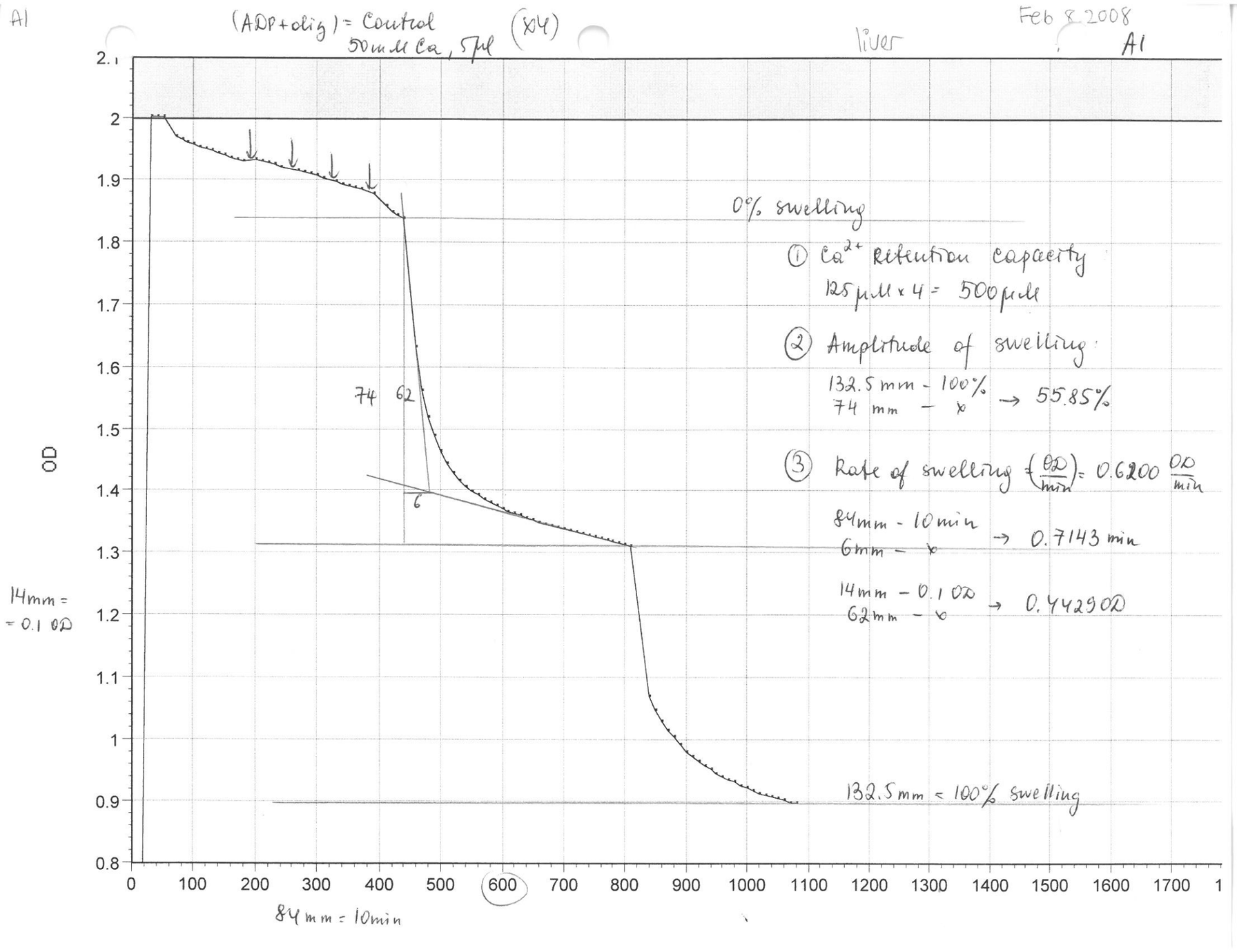
LIVER


ADP + Oligo + FCF2 + Cyclo A → 250 mM Ca^{2+} (5 µl/adding)
liver
A7 (RLM)
Jan. , 2008
0% swelling
① Ca retention capacity:
625 µM x 5 = 3125 µM = 3.125 mM
② Amplitude of swelling:
134.5 – 100%
71.5 – x → 53.16%
③ Rate of swelling:
84 mm – 10 min
9.5 – x → 1.13 min
14 mm = 0.1 OD
51.5 – b → 0.3679 OD
Rate of swelling = 0.3255 OD/min
134.5 mm = 100% swelling
51.5
71.5
9.5
OD
14mm = 0.1 OD
84 mm = 10 min

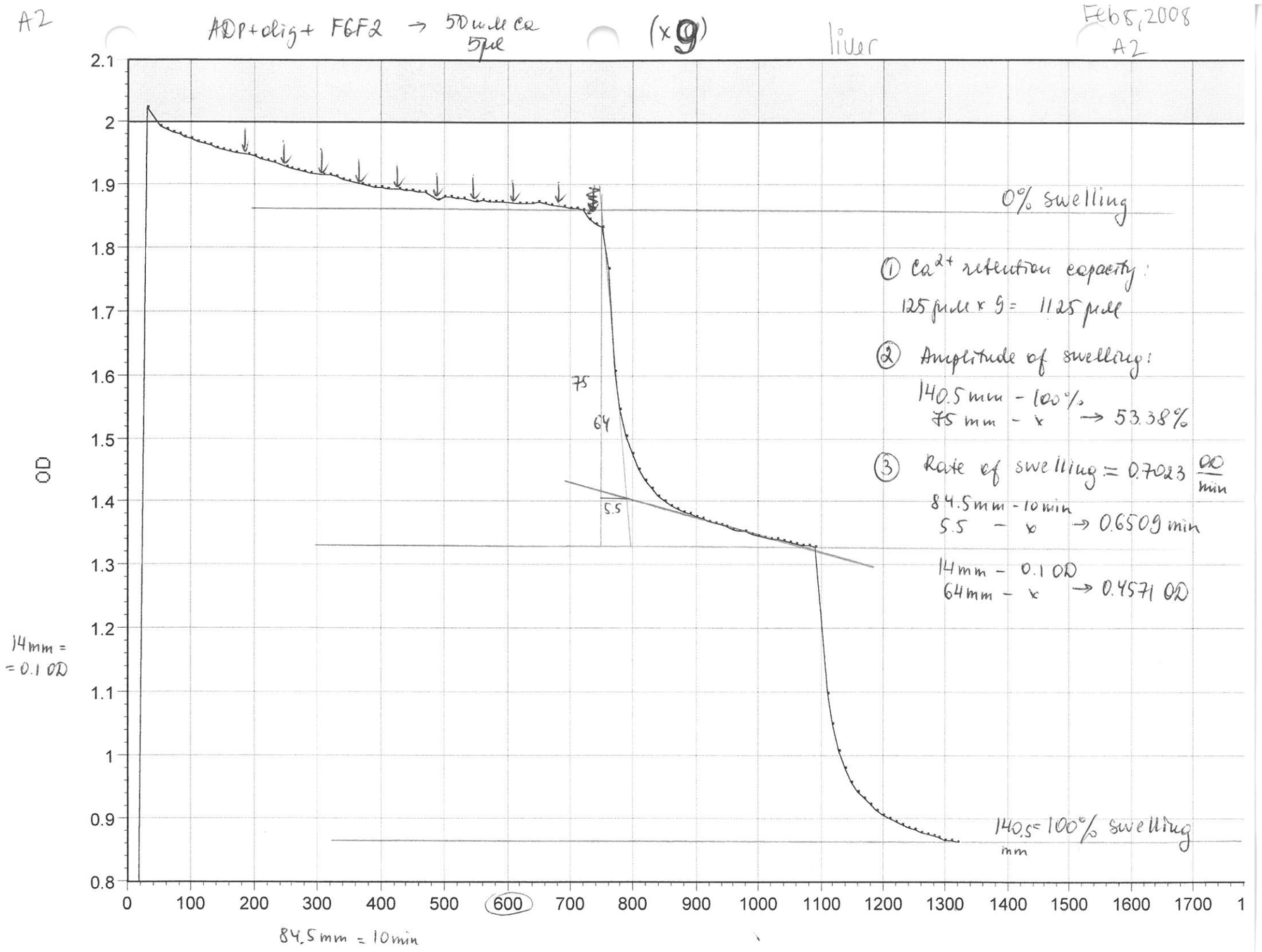
LIVER


ADP + Oligo → 50 mM Ca^{2+}
15 µl (adding)
liver
A8 (RLM)
Jan . , 2008
OD
0% swelling
73 63
9.5
① Ca^{2+} retention capacity:
125 µM x 2 = 250 µM
② Amplitude of swelling:
152 mm – 100%
73 mm – x → 48.03%
③ Rate of swelling:
85 mm – 10 min
9.5 – x → 1.1176 min
14 mm – 0.1 OD
63 mm – x → 0.45 OD
Rate of swelling = 0.4026 OD/min
14mm = = 0.1 OD
152 = 100% swelling.
85 mm – 10 min

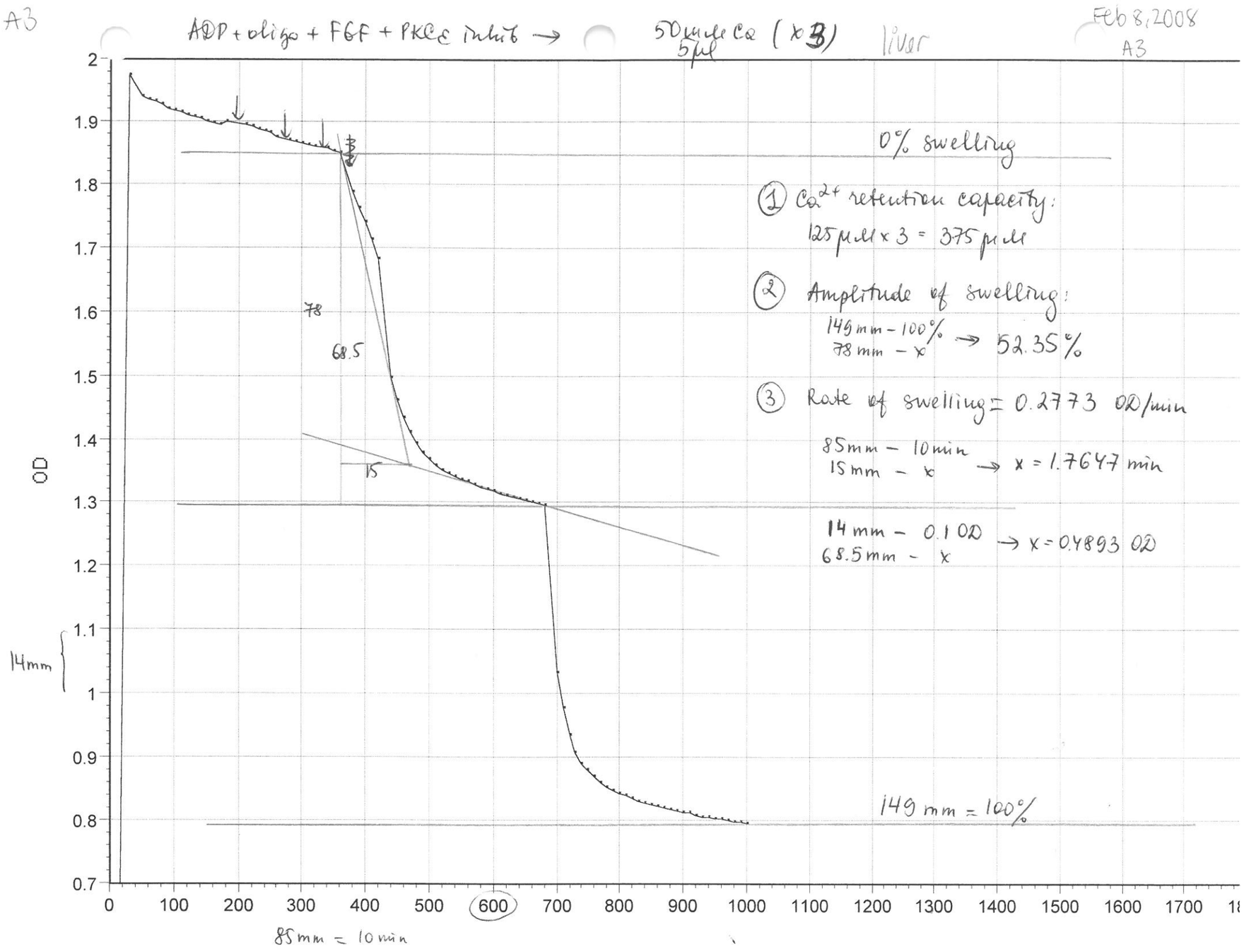
LIVER


A1
(ADP+olig) = Control
50mM Ca, 5µl
(X4)
liver
Feb 8 2008
A1
OD
2.1
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
74
62
6
0% swelling
① Ca2+ Retention capacity
125 µM x 4 = 500 µM
② Amplitude of swelling:
132.5 mm - 100%
74 mm - x → 55.85%
③ Rate of swelling (ΔOD/min) = 0.6200 OD/min
84mm - 10 min
6mm - x → 0.7143 min
14mm - 0.1 OD
62mm - x → 0.44290D
132.5mm = 100% swelling
14mm = = 0.1 OD
84 mm = 10min

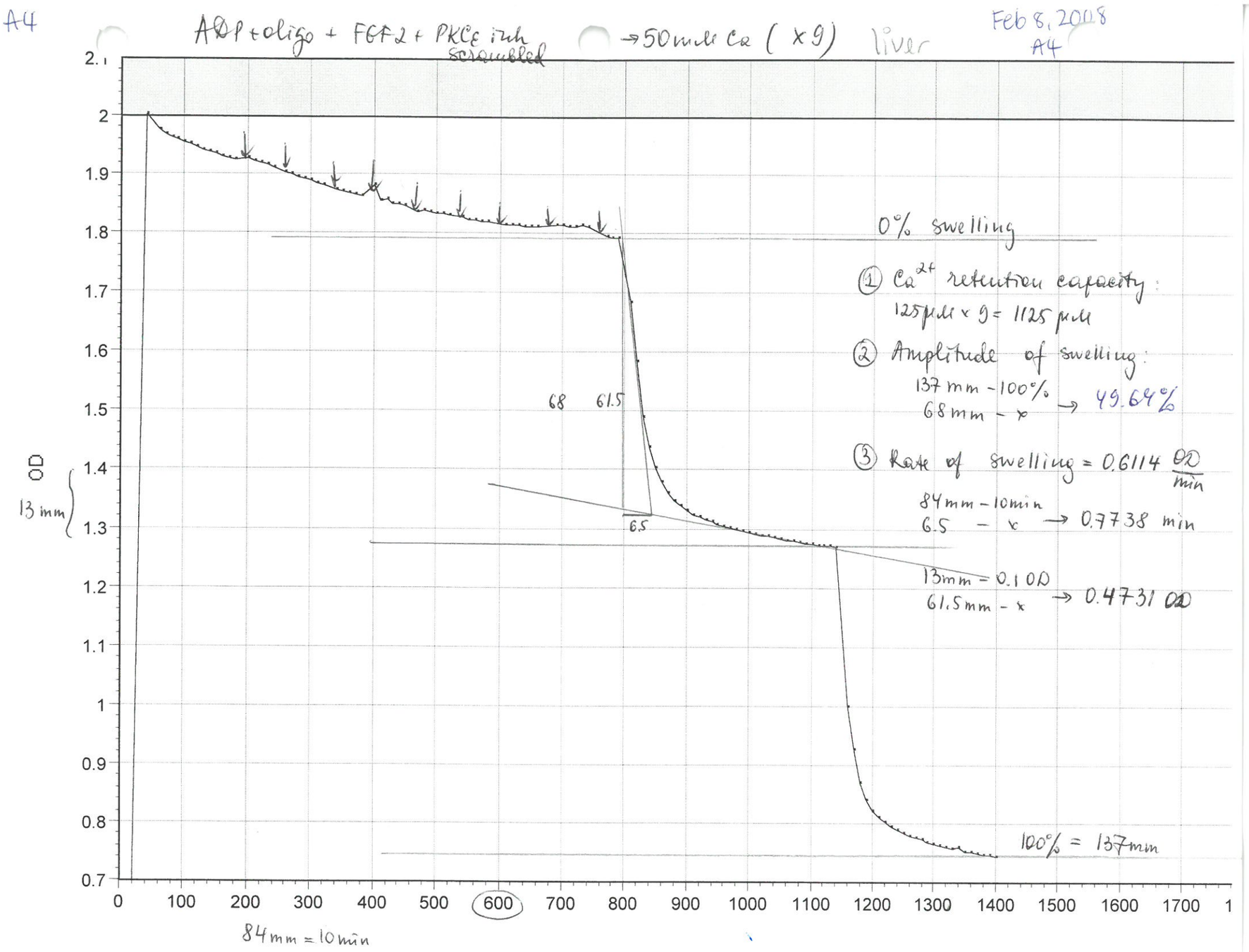
LIVER


A2
ADP+dig+ FGF2 → 50mM Ca 5μl
(x g)
liver
Feb 5, 2008
A2
OD
0% swelling
① Ca2+ retention capacity:
125 μM x 9 = 1125 μM
② Amplitude of swelling:
140.5 mm - 100%
75 mm - x → 53.38%
③ Rate of swelling = 0.7023 OD/min
84.5 mm - 10 min
5.5 - x → 0.6509 min
14 mm - 0.1 OD
64 mm - x → 0.4571 OD
75
64
5.5
14mm = = 0.1 OD
140.5 mm = 100% swelling
84.5 mm = 10 min

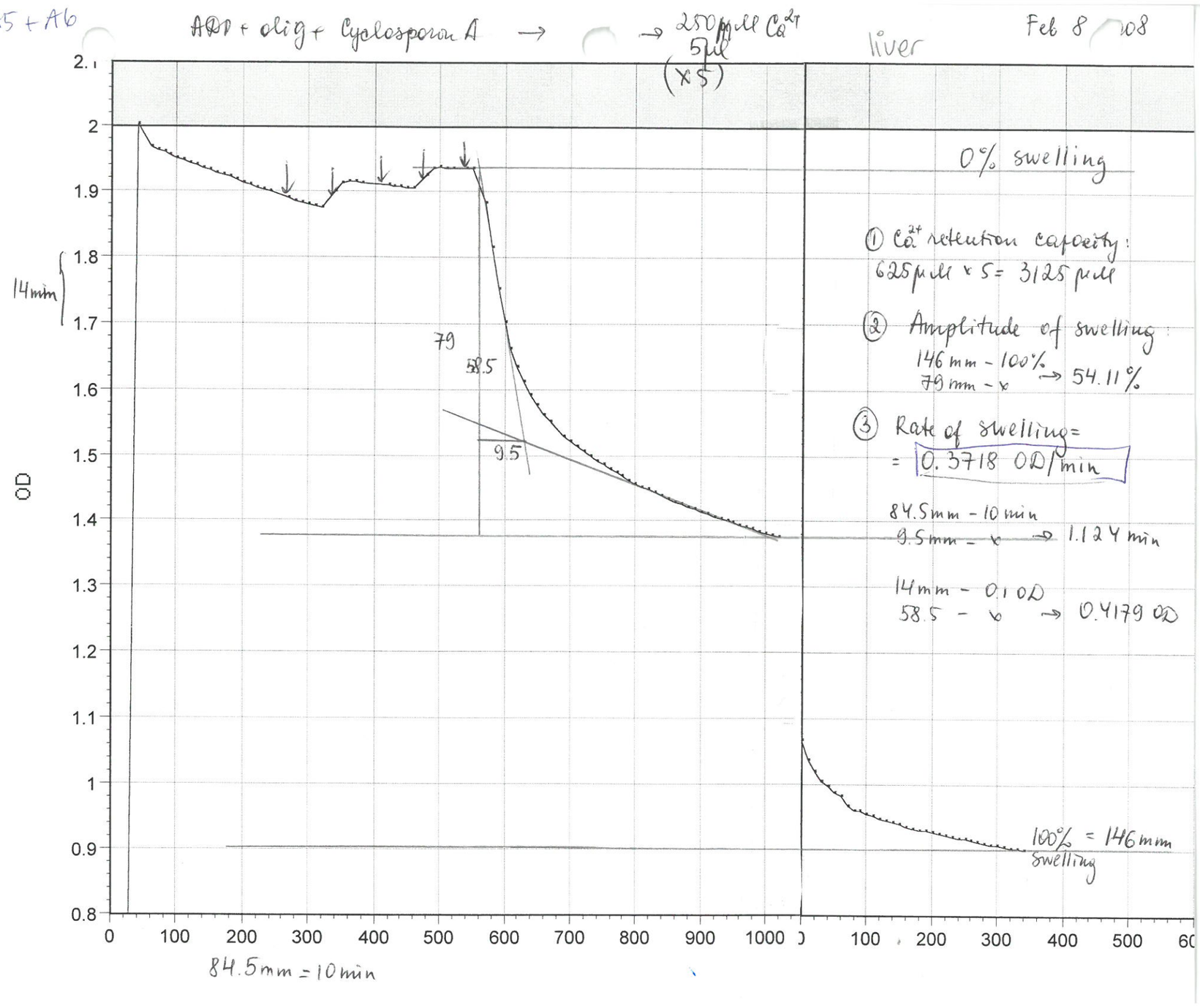
LIVER


A3
ADP + oligo + FGF + PKCε inhib → 50μg/ml Ca (x3) liver
5μl
Feb 8, 2008
A3
OD
14mm
0% swelling
① Ca^{2+} retention capacity:
125 μl x 3 = 375 μl
② Amplitude of swelling:
149 mm - 100%
78 mm - x → 52.35%
③ Rate of swelling = 0.2773 OD/min
85mm - 10min
15mm - x → x = 1.7647 min
14 mm - 0.1 OD
68.5 mm - x → x = 0.4893 OD
78
68.5
15
149 mm = 100%
85mm = 10min

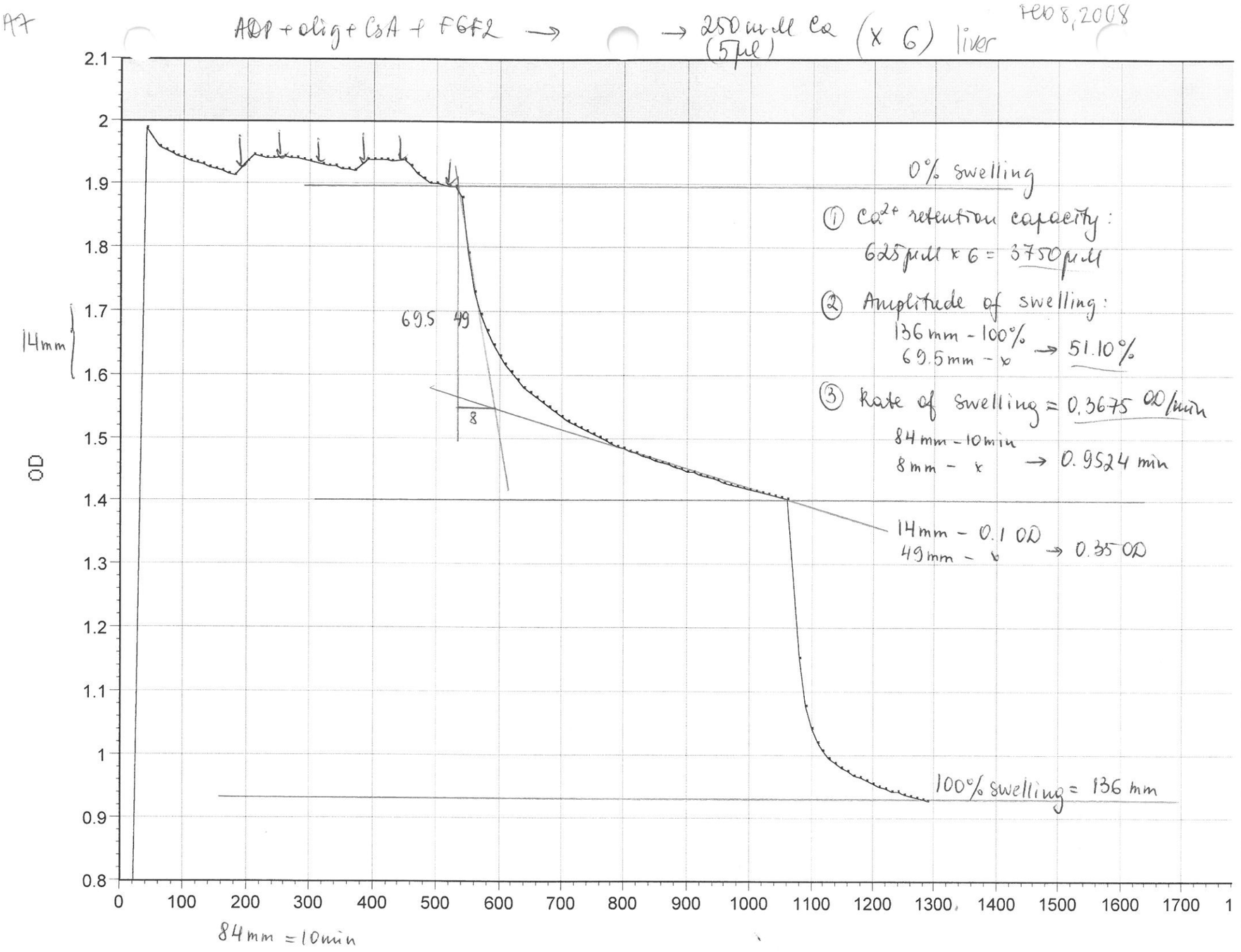
LIVER


A4
ADP + oligo + FGF2 + PKCε inh scrambled → 50mM Ca (x9) liver
Feb 8, 2008
A4
OD
13 mm
0% swelling
① Ca2+ retention capacity:
125μM x 9 = 1125 μM
② Amplitude of swelling:
137 mm - 100%
68 mm - x → 49.64%
68
61.5
③ Rate of swelling = 0.6114 OD/min
84mm - 10min
6.5 - x → 0.7738 min
6.5
13mm = 0.1 OD
61.5mm - x → 0.4731 OD
100% = 137mm
84mm = 10min

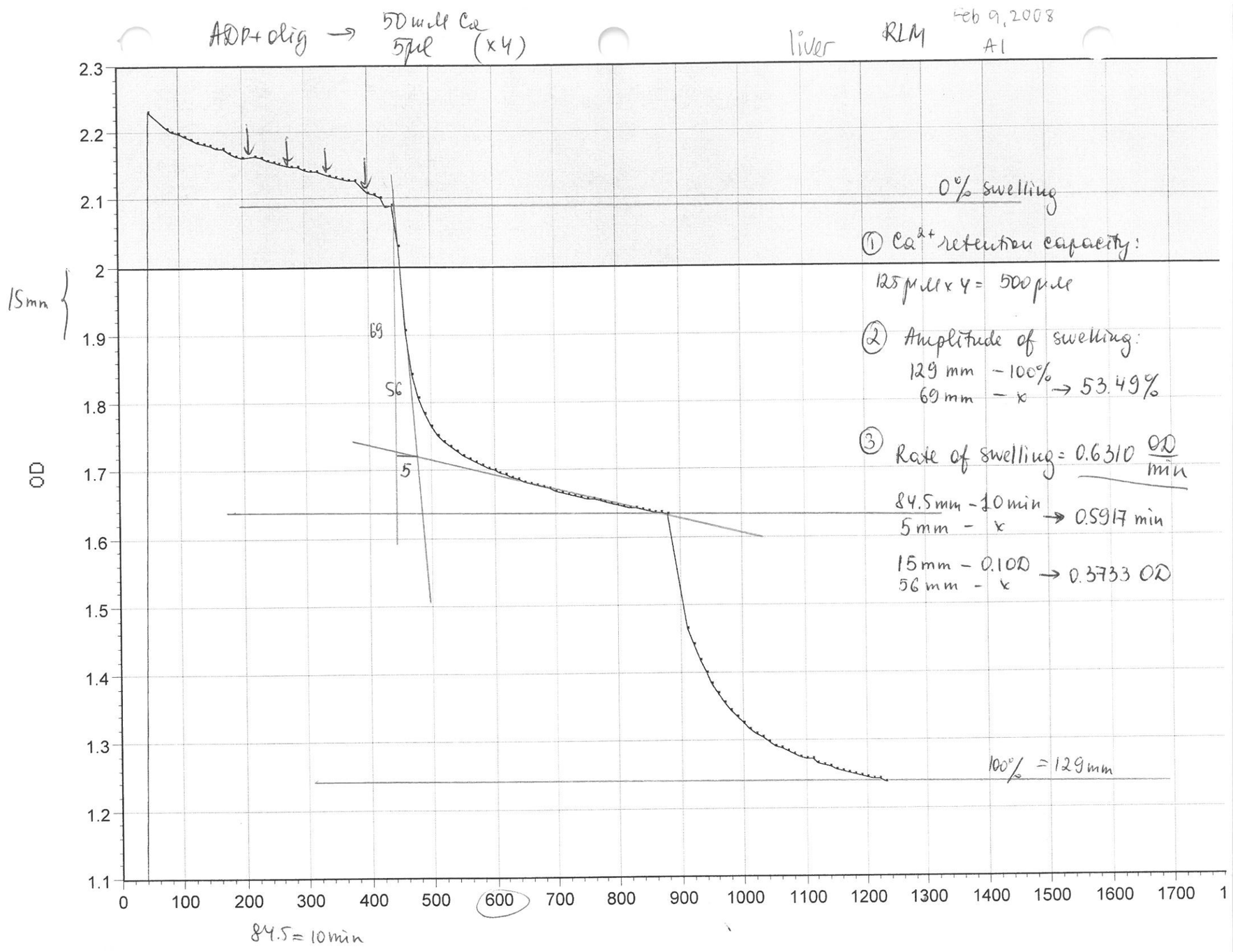
LIVER


A5 + A6
ADP + olig + Cyclosporin A → → 250 μM Ca2+ 5μl (x5)
liver
Feb 8 08
0% swelling
① Ca2+ retention capacity:
625 μM x 5 = 3125 μM
② Amplitude of swelling:
146 mm - 100%
79 mm - x → 54.11%
③ Rate of swelling =
= 0.3718 OD/min
84.5 mm - 10 min
9.5 mm - x → 1.124 min
14 mm - 0.1 OD
58.5 - x → 0.4179 OD
100% = 146 mm swelling
14 mm
OD
79
58.5
9.5
84.5 mm = 10 min

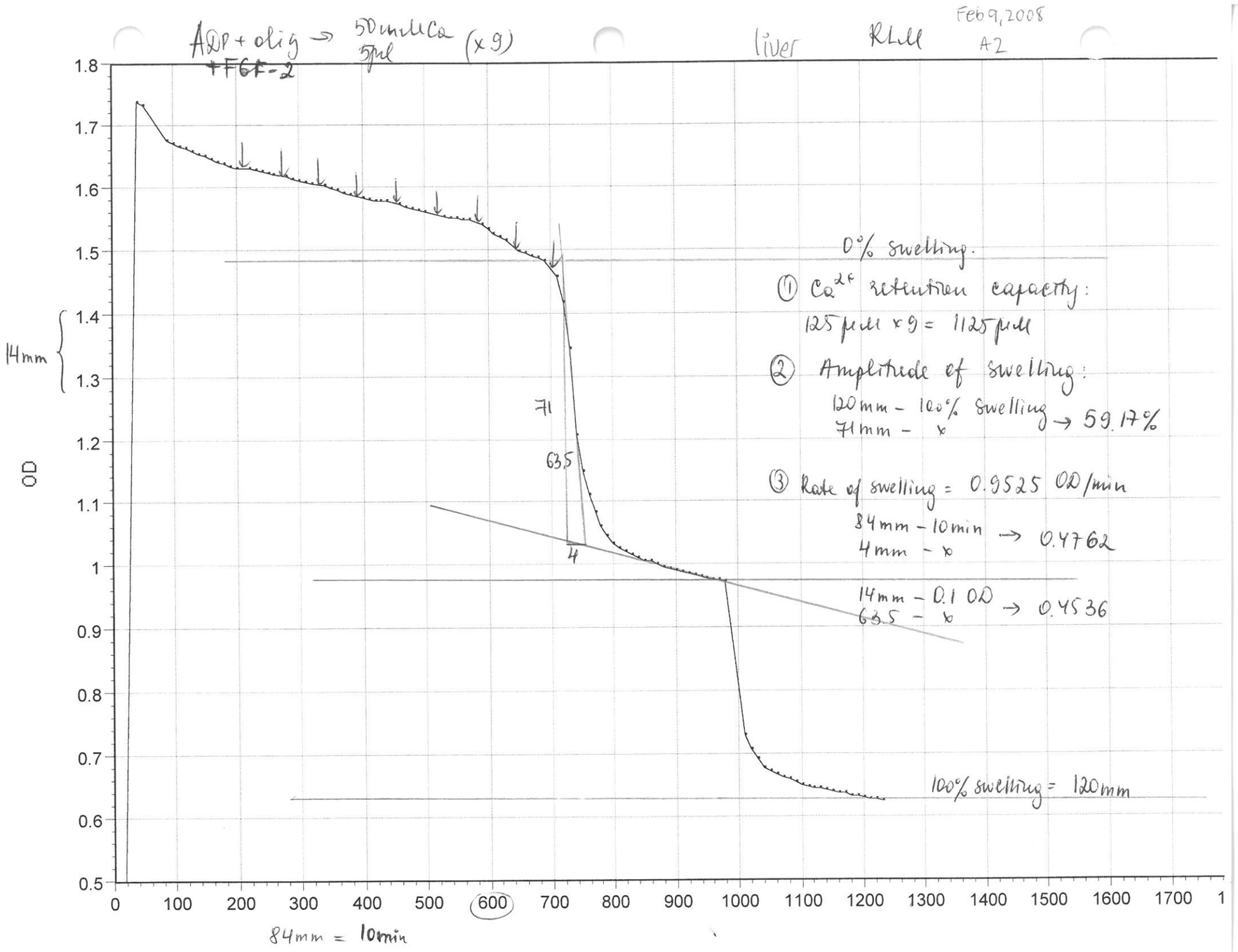
LIVER


A7
ADP + olig + CsA + FGF2 →
→ 250 mM Ca (5μl) (x 6) liver
Feb 8, 2008
OD
14mm
69.5
49
8
0% swelling
① Ca2+ retention capacity:
625 μM x 6 = 3750 μM
② Amplitude of swelling:
136 mm - 100%
69.5 mm - x → 51.10%
③ Rate of swelling = 0.3675 OD/min
84 mm - 10 min
8 mm - x → 0.9524 min
14mm - 0.1 OD
49mm - x → 0.35 OD
100% swelling = 136 mm
84mm = 10min

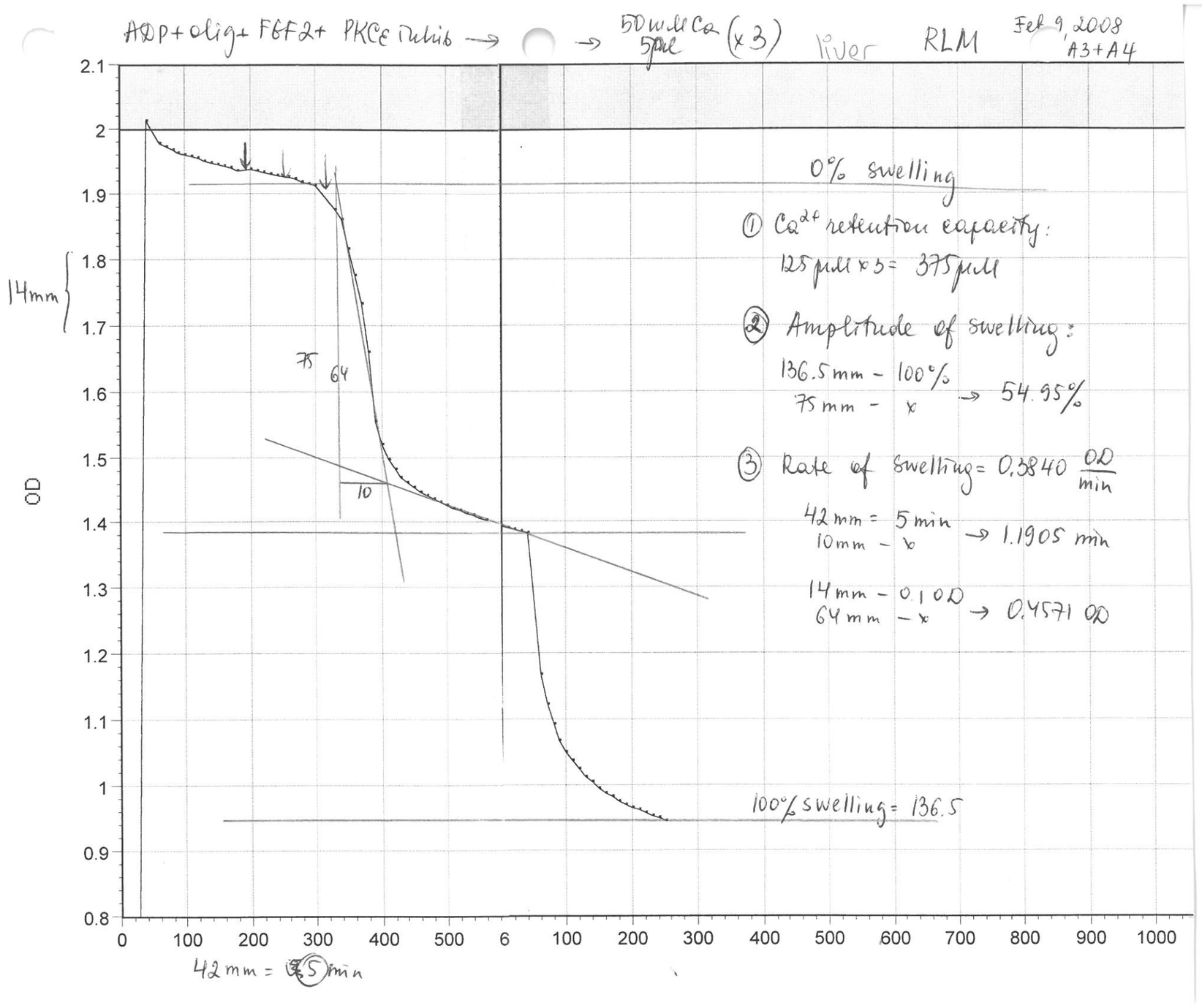
LIVER


ADP+olig → 50 mM Ca 5µl (x4)
liver RLM
Feb 9, 2008
A1
0% swelling
① Ca2+ retention capacity:
125 µM x 4 = 500 µM
② Amplitude of swelling:
129 mm – 100%
69 mm – x → 53.49%
③ Rate of swelling = 0.6310 OD/min
84.5 mm – 10 min
5 mm – x → 0.5917 min
15 mm – 0.10D
56 mm – x → 0.3733 OD
100% = 129 mm
15mn
OD
69
56
5
84.5 = 10 min

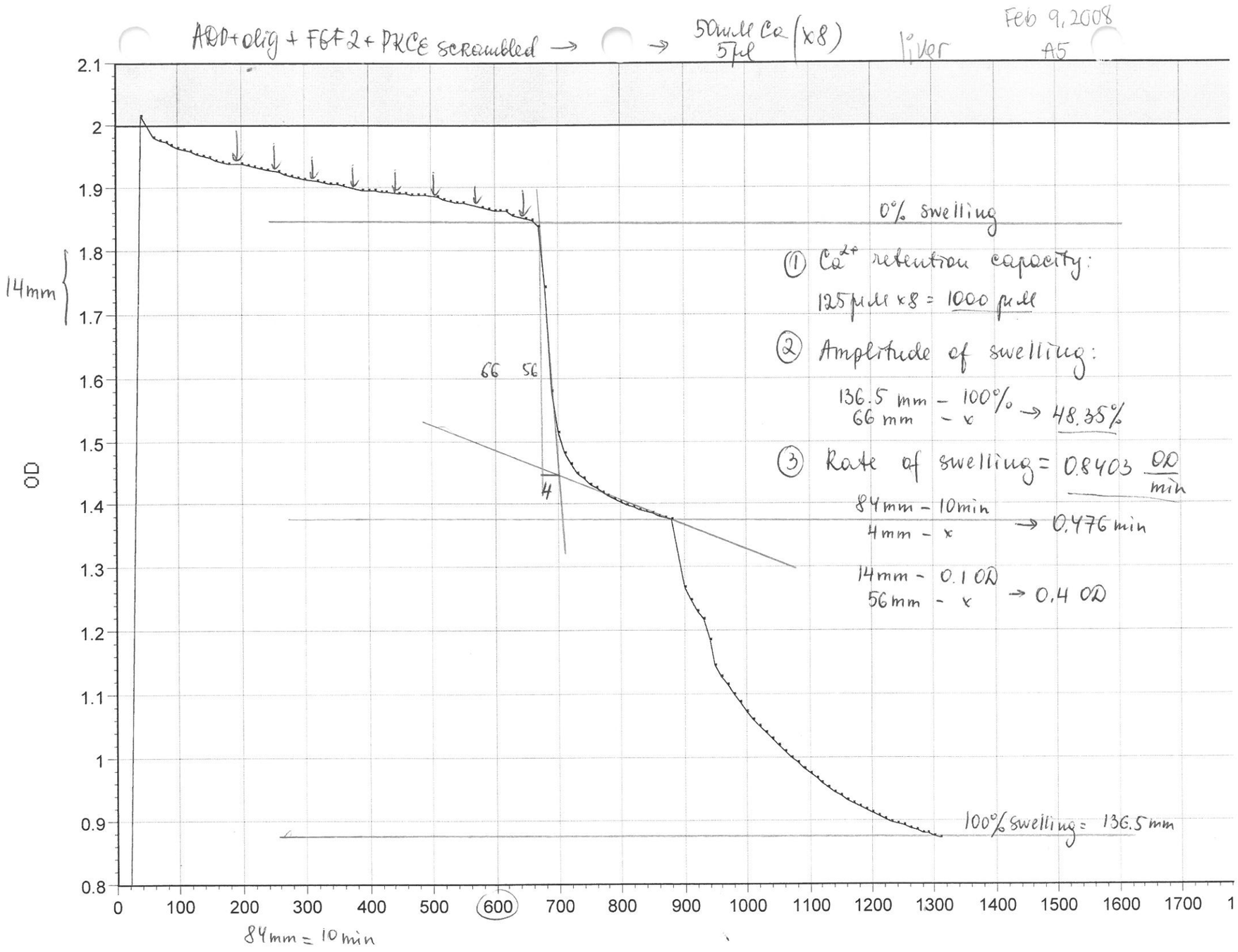
LIVER


ADP + olig → 50mM Ca 5μl (x9)
+FGF-2
liver RLll
Feb 9, 2008
A2
OD
14mm
71
63.5
4
0% swelling.
① Ca2+ retention capacity:
125 μl x 9 = 1125 μl
② Amplitude of swelling:
120mm - 100% swelling
71mm - x
→ 59.17%
③ Rate of swelling = 0.9525 OD/min
84mm - 10min → 0.4762
4mm - x
14mm - 0.1 OD
63.5 - x
→ 0.4536
100% swelling = 120mm
84mm = 10min

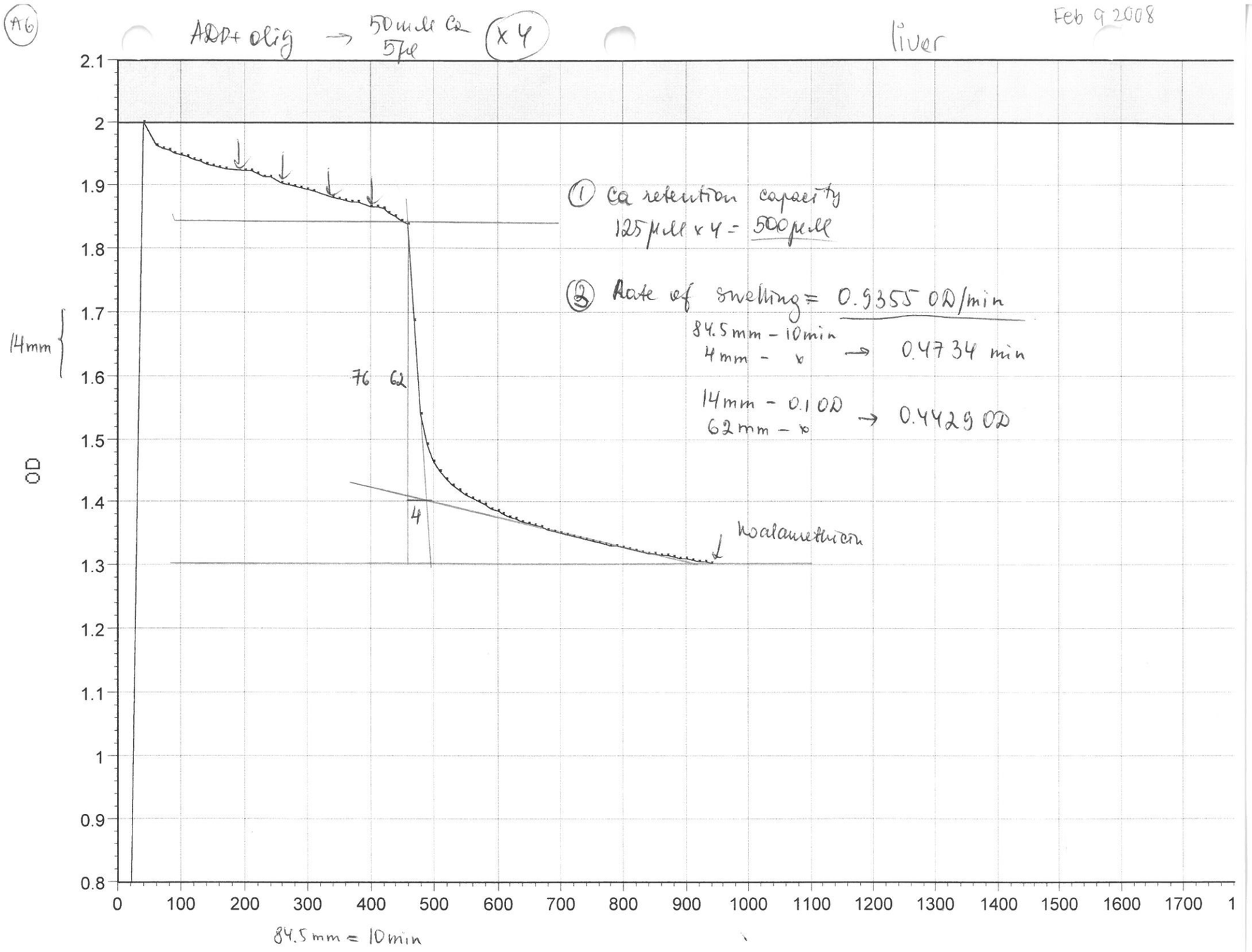
LIVER


ADP+olig+ FGF2+ PKCε inhib →
→ 50 mM Ca 5 µl (x3)
liver RLM
Feb 9, 2008
A3+A4
OD
14mm
75
64
10
0% swelling
① Ca2+ retention capacity:
125 µM x 5 = 375 µM
② Amplitude of swelling:
136.5 mm - 100%
75 mm - x → 54.95%
③ Rate of swelling = 0.3840 OD/min
42 mm = 5 min
10 mm - x → 1.1905 min
14 mm - 0.1 OD
64 mm - x → 0.4571 OD
100% swelling = 136.5
42 mm = 5 min

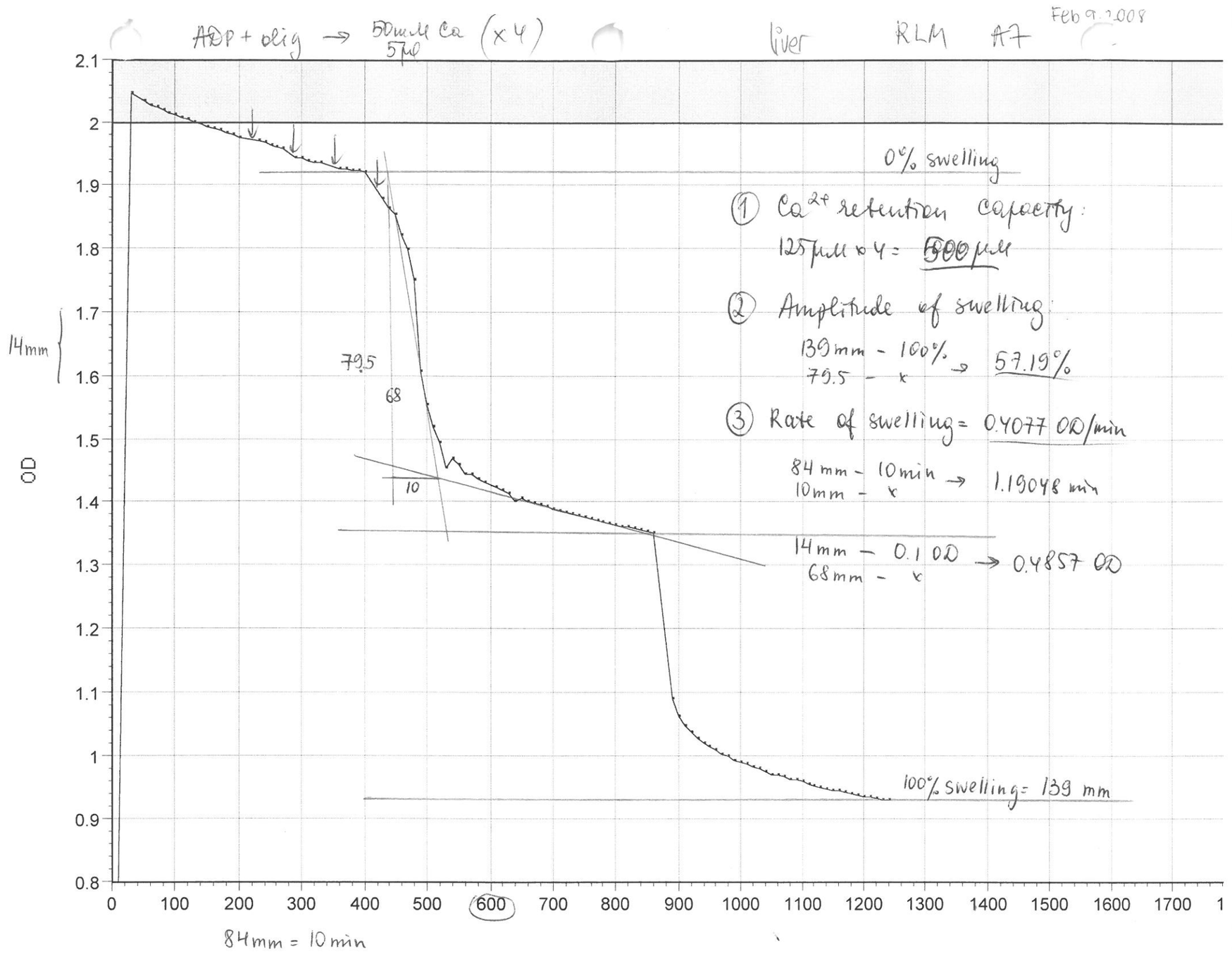
LIVER


ABD+olig + FGF2+ PKCε scrambled → → 50mM Ca 5μl (x8)
liver
Feb 9, 2008
A5
OD
14mm
66 56
4
0% swelling
① Ca2+ retention capacity:
125μM x8 = 1000μM
② Amplitude of swelling:
136.5 mm – 100%
66 mm – x → 48.35%
③ Rate of swelling = 0.8403 OD/min
84mm – 10min
4mm – x → 0.476 min
14mm – 0.1 OD
56mm – x → 0.4 OD
100% swelling = 136.5mm
84mm = 10min

LIVER

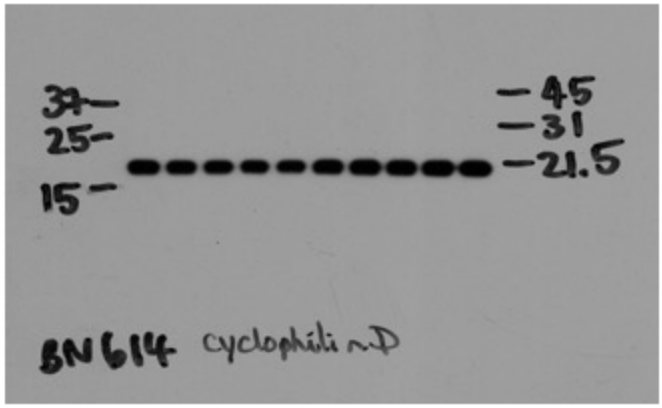

A6
ADP + olig → 50mM Ca 5μl (x4)
liver
Feb 9 2008
2.1
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
OD
14mm
76 62
4
Incalamethicin
① Ca retention capacity
125 μM x 4 = 500 μM
② Rate of swelling = 0.9355 OD/min
84.5mm - 10min
4mm - x → 0.4734 min
14mm - 0.1 OD
62mm - x → 0.4429 OD
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
84.5mm = 10min

LLIVER


ADP + olig → 50mM Ca (x4)
5μl
liver RLM A7
Feb 9, 2008
0% swelling
① Ca2+ retention capacity:
125μM x 4 = 500μM
② Amplitude of swelling:
139mm - 100%
79.5 - x → 57.19%
③ Rate of swelling = 0.4077 OD/min
84mm - 10min
10mm - x → 1.1904 min
14mm - 0.1 OD
68mm - x → 0.4857 OD
100% swelling = 139 mm
14mm
79.5
68
10
OD
84mm = 10min

Original blots for Fig.3

Fig.3a



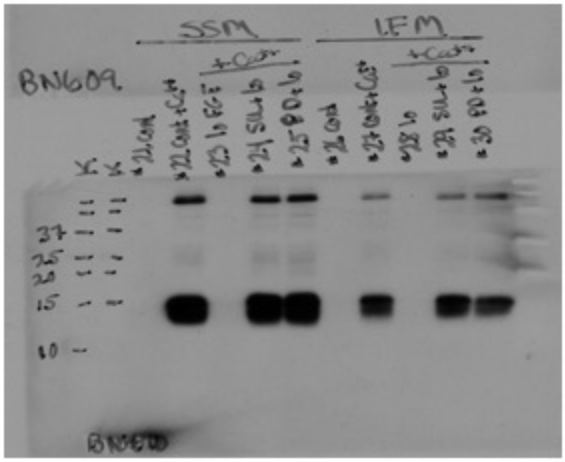
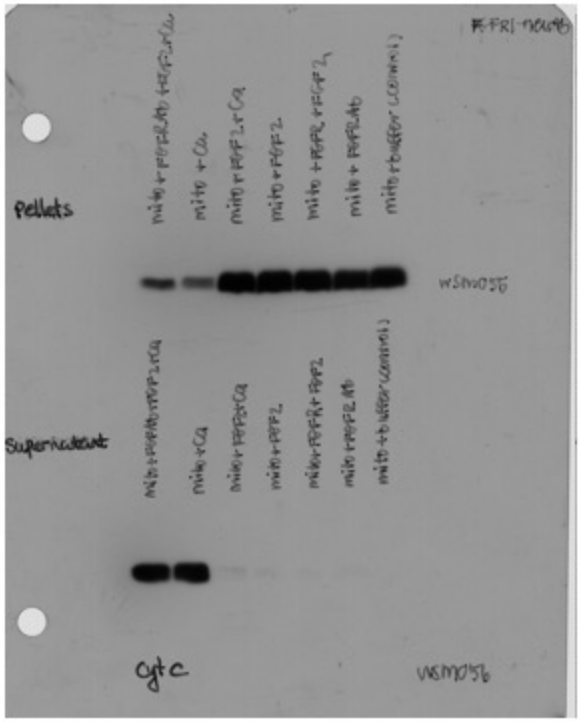
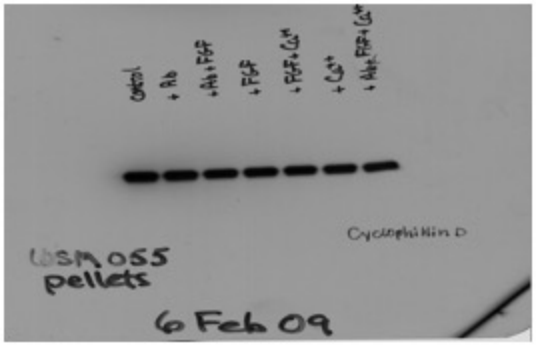


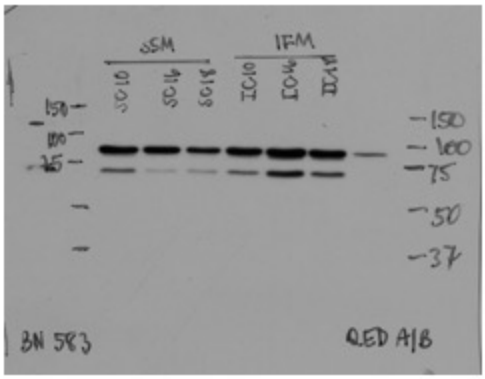
Fig.3b



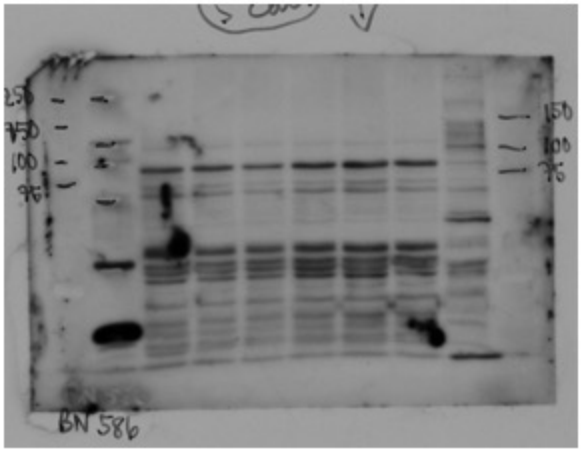


Original blots for Fig.2a

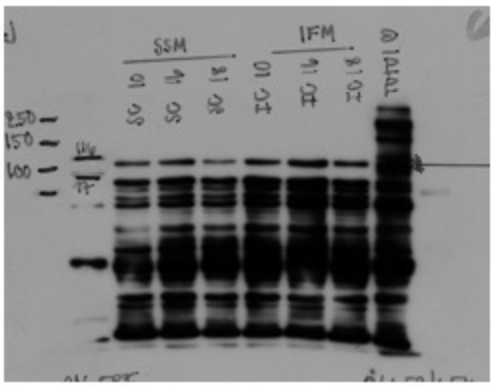
x anti-FGFR1, QED A/B.



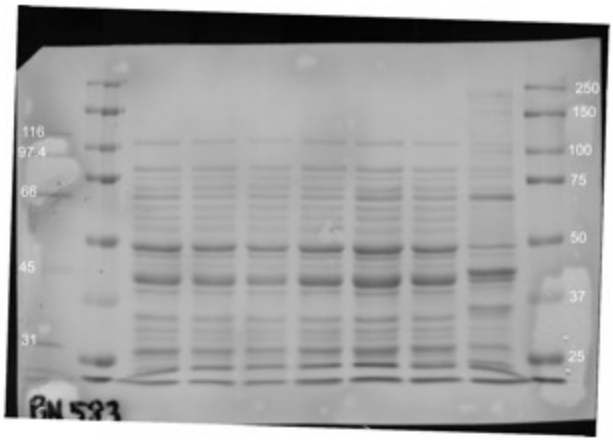
x anti-pY766-FGFR1



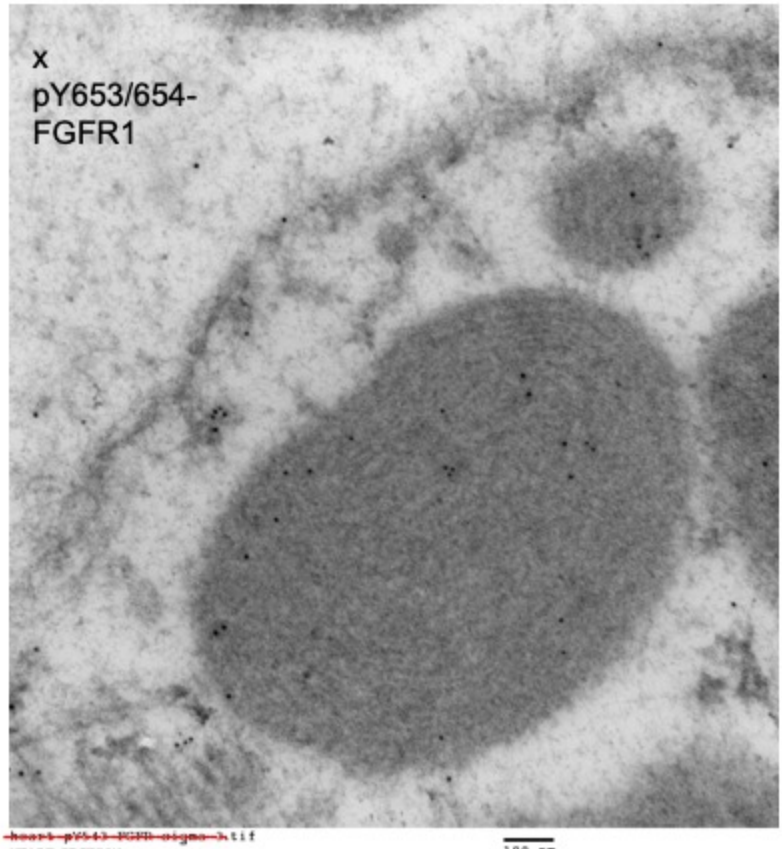
x anti-pY653/654-FGFR1.



Ponceau S stain

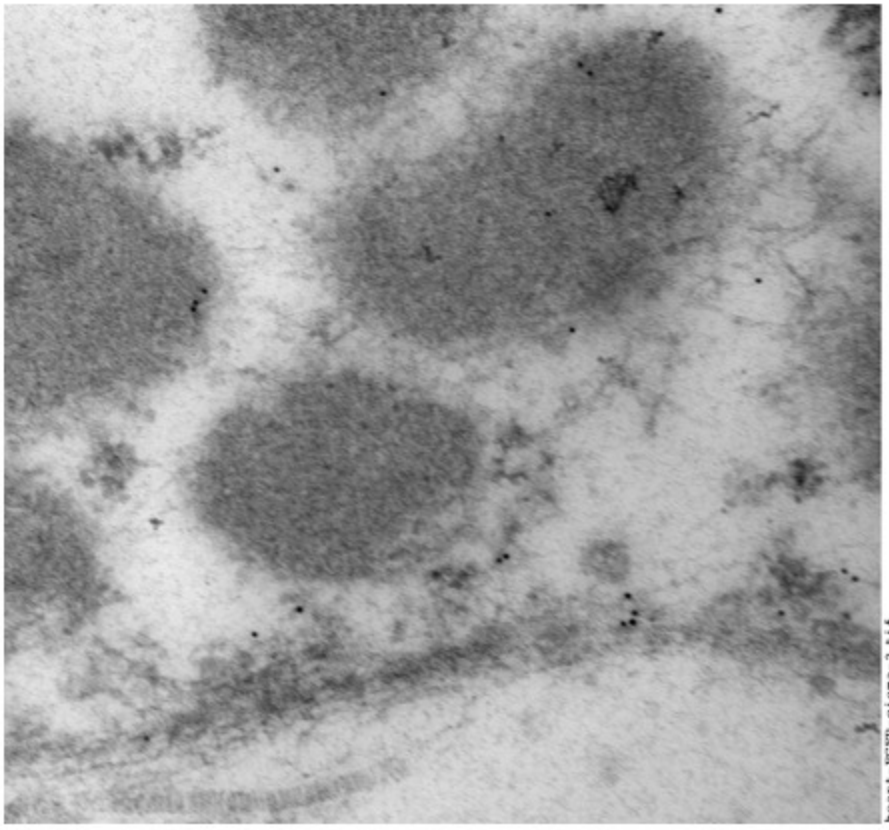


Original images for Fig.2b



Mislabeled- ~~heart-pY653-FGFR-sigma-3~~.tif
HEART SECTION
Print Mag: 116000x @ 7.0 in
11:57 10/31/08
ito

100 nm
HV=80.0kV
Direct Mag: 100000x
Facility of Medicine EM Unit



heart-FGFR-sigma-2.tif
HEART SECTION
Print Mag: 140000x @ 7.0 in
11:26 10/31/08
ito

100 nm
HV=80.0kV
Direct Mag: 120000x
Facility of Medicine EM Unit

Original blots for Fig.4

Fig.4a

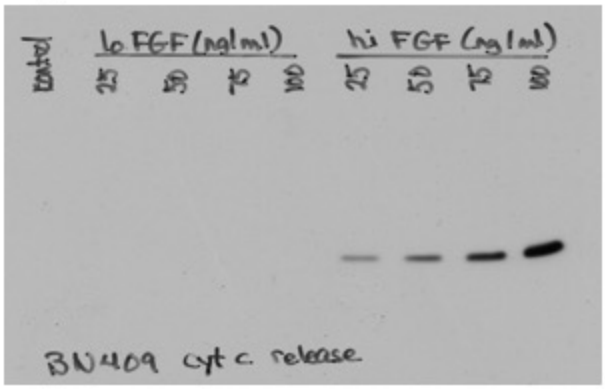


Fig.4b

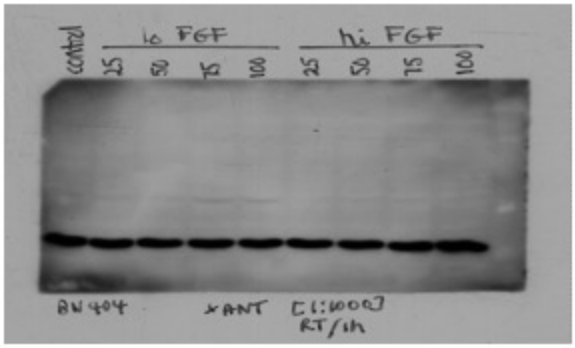


Fig.4c

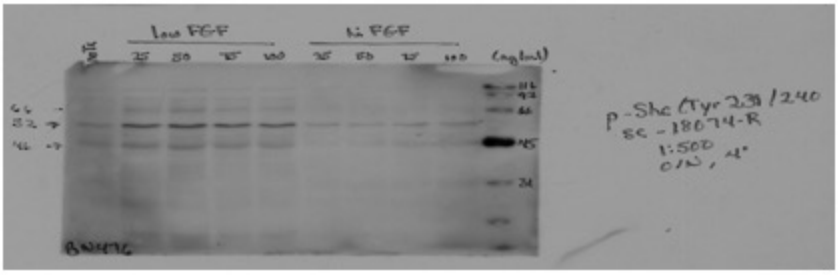


Fig.4d

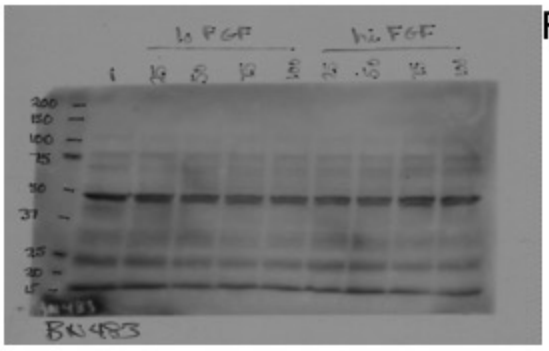
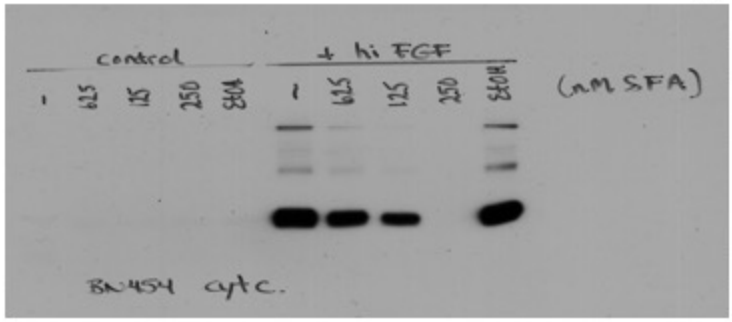


Fig.4e



Original blots for Fig.5

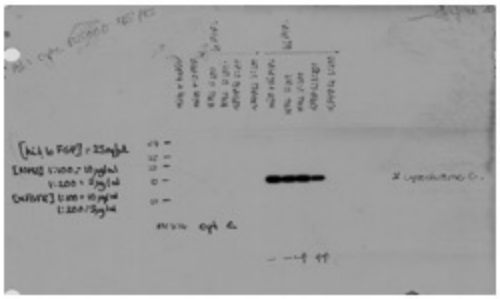


Fig.5a

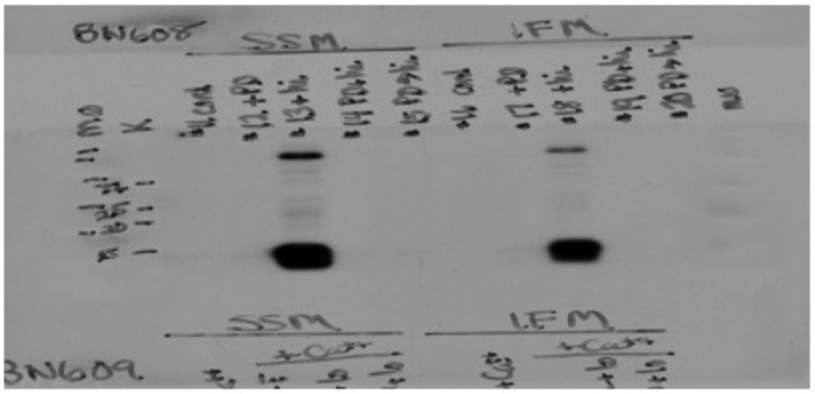


Fig.5b

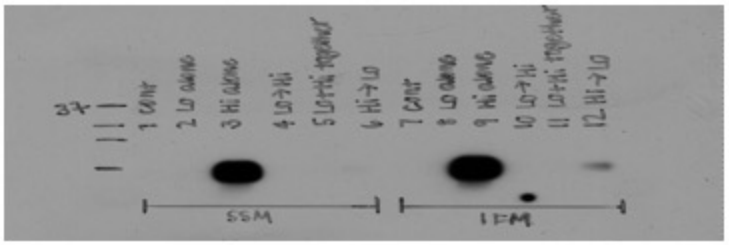
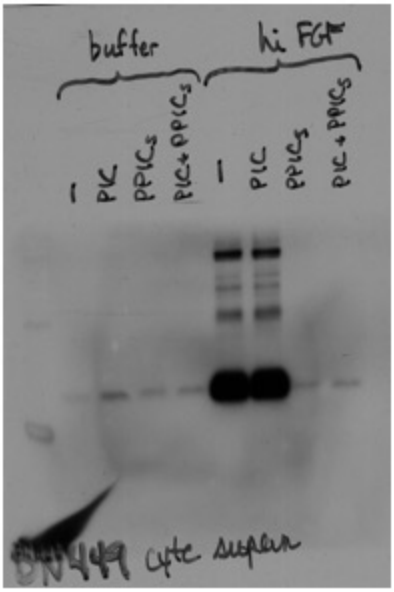
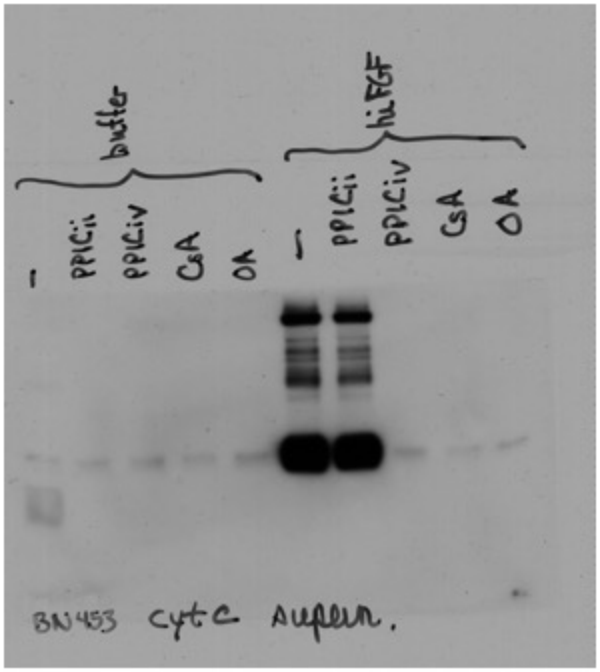


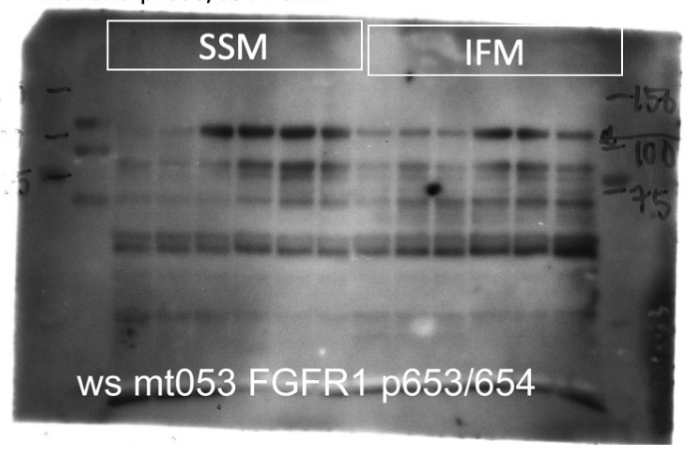
Fig.5c

Fig.5e

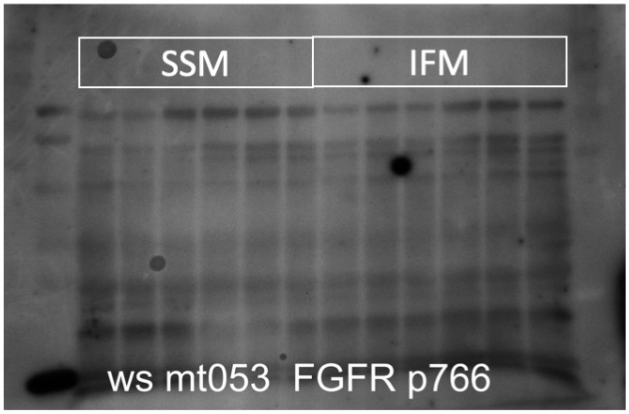




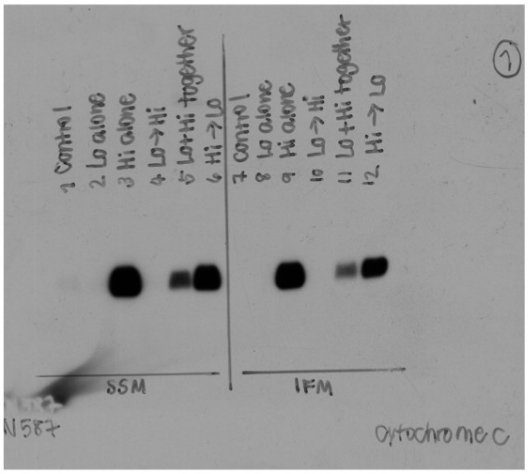
Repeat experiment: SSM and IFM preparations probed with anti-pY653/654-FGFR1

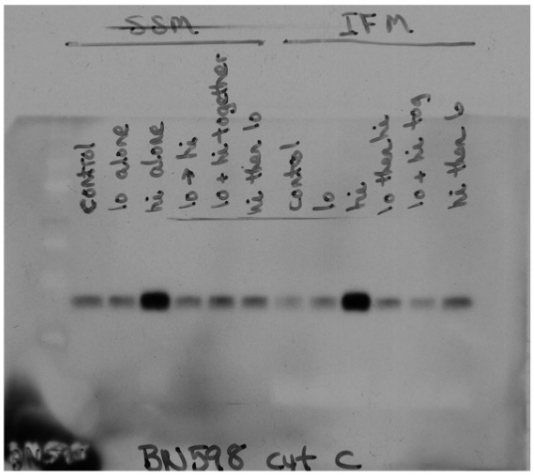


Repeat experiment: SSM and IFM preparations probed with anti-pY653/654-FGFR1

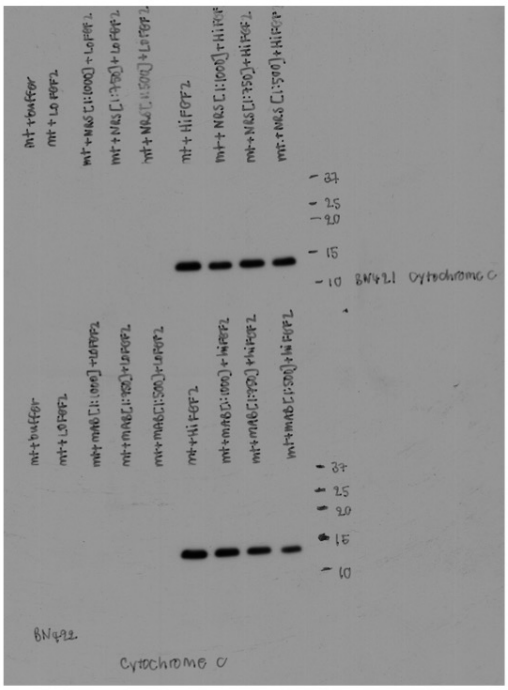


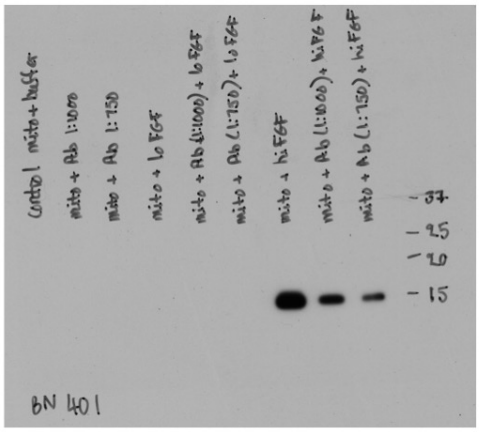
Repeat experiments: Western blots showing supernatant cytochrome c after exposure to Hi-FGF2, and Lo-FGF2

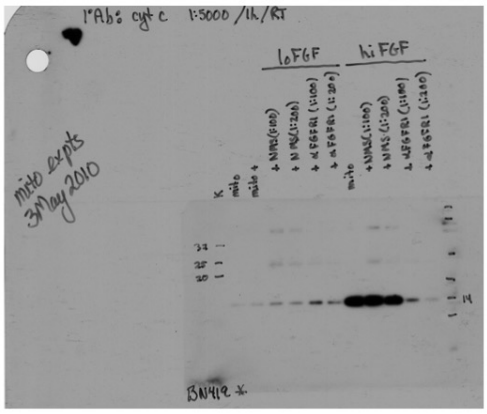




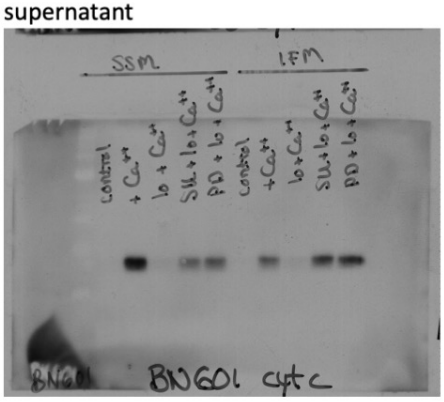
Repeat experiments: Western blots showing supernatant cytochrome c after exposure to Hi-FGF2 in the presence of non-immune antibodies (upper gel) or neutralizing antibodies (lower gel)



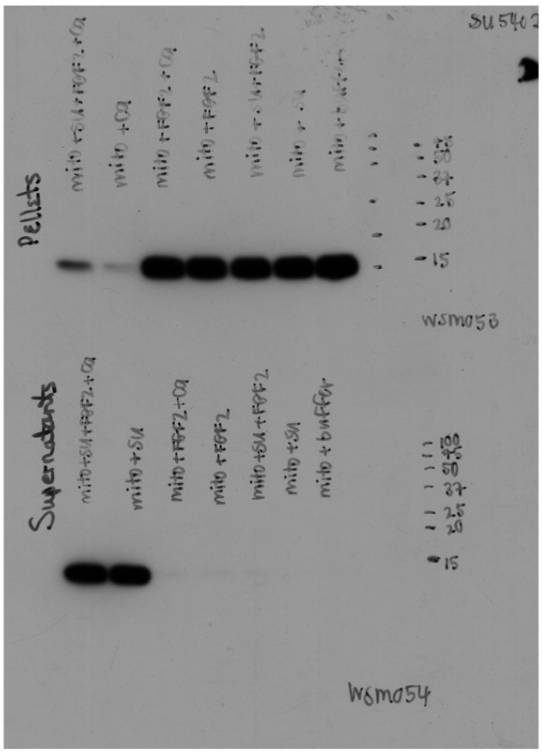




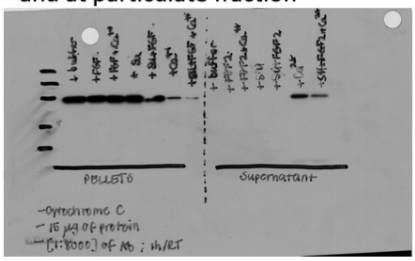
Repeat experiment: SSM and IFM treated with Lo-FGF2 in the presence and absence of FGFR1 inhibitors (SU-, PD-) and subjected to calcium overload: Cyt-C at supernatant



Repeat experiment: SSM treated with Lo-FGF2 in the presence and absence of FGFR1 inhibitor SU-, and subjected to calcium overload: Cyt-C at supernatant and at particulate fraction



Repeat experiment: SSM treated with Lo-FGF2 in the presence and absence of FGFR1 inhibitor SU-, and subjected to calcium overload: Cyt-C at supernatant and at particulate fraction



Repeat experiments: Western blots of SSM and IFM treated with Hi-FGF2 in the presence and absence of FGFR1 inhibitor PD-, SU-: Cyt-C at supernatant