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APPENDICES

CALCULATION OF THE DATA

1. Coefficient of determination

    least squares method :-

    $$r^2 \;=\; \frac{[n\Sigma(xy) - \Sigma x \Sigma y]^2}{[n\Sigma x^2 - (\Sigma x)^2][n\Sigma y^2 - (\Sigma y)^2]}$$

2. Specific rate constant (k)

    $$k \;=\; \frac{n\Sigma(xy) - \Sigma x \cdot \Sigma y}{n\Sigma x^2 - (\Sigma x)^2}$$

    (k : were calculated from the slope of linear line)

    $$k \; sk \;=\; k \cdot sy/x \sqrt{\frac{1}{\Sigma(x - \bar{x})^2}}$$

    where :-

    x   =   time (day)

    y   = $\ln A/A_o$

3. The extrapolated values of k at room temperature (33^oC)

    $$\ln k \;=\; \ln A - \frac{\triangle Ha}{R} \cdot \frac{1}{T}$$

calculated the specific rate constant (k) at room temperature by substituted the T value with 306 in the above equation

$$k \pm sk = k \pm sy/x \sqrt{1 + \frac{1}{n} + \frac{(x_o - \bar{x})^2}{x^2 - \frac{(\sum x)^2}{n}}}$$

where :-

x_o = the extrapolated specific rate constant at room temperature

x = average specific rate constant of 40°, 50°, 60°, and 70^oC.

n = the observed values (= 4)

Table 1 Phase Solubility Data for Chloramphenicol:β-CD in Water at Room Temperature.

Molarity of β-CD ($\times 10^2 M$)	Molarity of Chloramphenicol ($\times 10^2 M$)
0.00	1.68
0.443	1.98
1.06	2.35
1.32	2.53
2.20	3.10
2.64	3.36
3.08	3.60
3.40	3.83
3.51	3.91
3.57	3.93
3.66	3.93
3.97	3.93
4.84	3.92
5.59	3.93
5.73	3.92
5.80	3.79
5.95	3.70
6.39	3.41
6.83	3.03
7.27	2.78
7.49	2.70
8.37	2.50
8.81	2.58
9.69	2.67

Table 2   The Values of ln A/A_O of Chloramphenicol, Comparing
the Freezed Method (F) with Non-Freezed Method (NF).

Day	ln A/A_O^a	
	F	NF
0.000	4.5221	4.5536
1.010	4.4590	4.4520
2.054	4.2908	4.3594
2.960	4.2370	4.2346
4.060	4.1376	4.1376
5.054	3.9641	4.0086
6.064	3.8520	3.9565

ln A/A_O : The logarithmic values of the content remained of
chloramphenicol (A) to the initial chloramphenicol (A_O)

a : average from three values (n = 3).

Table 3 The Values of ln A/A_o of the Complex, Comparing
the Freezed Method (F) with the Non-Freezed Method (NF).

Day	ln A/A_o [a]	
	F	NF
0.000	4.5421	4.5566
1.010	4.5089	4.5255
2.054	4.4650	4.4889
2.960	4.4314	4.4494
4.060	4.3904	4.4184
5.054	4.3548	4.3720
6.064	4.3034	4.3366

ln A/A_o : The logarithmic values of the remained content of
complex (A) to the initial complex (A_o).

a : average from three values (n = 3).

Table 4   Data of Chloramphenicol Content Remained at 70°,60°,50°, 40°C and at Room Temperature (33°C).

70°C [a]			60°C [a]			50°C [a]			40°C [a]			Room Temp(30°-35°) [a]		
day	%A/A₀	ln A/A₀	day	%A/A₀	ln A/A₀	day	%A/A₀	ln A/A₀	day	%A/A₀(%)	ln A/A₀	day	%A/A₀	ln A/A₀
0.000	92.35	4.5256	0.000	89.98	4.4995	0.000	96.18	4.5663	0.000	101.6	4.6210	0.000	99.90	4.6042
1.000	86.49	4.4600	1.000	87.72	4.4741	1.000	95.79	4.5621	1.000	101.15	4.6166	7.000	98.96	4.5947
2.005	80.88	4.3930	2.005	85.50	4.4485	4.005	92.27	4.5247	3.007	99.98	4.6050	14.028	95.87	4.5630
3.007	74.25	4.3074	3.007	82.86	4.4172	7.000	88.49	4.4828	9.007	97.60	4.5809	21.010	96.15	4.5659
4.054	68.99	4.2340	5.028	78.45	4.3625	10.007	84.98	4.4424	15.000	95.15	4.5555	35.007	91.83	4.5199
5.028	63.08	4.1444	7.000	73.81	4.3015	12.976	81.94	4.4060	21.000	93.12	4.5339	50.000	91.02	4.5111
6.064	57.40	4.0500	10.007	66.98	4.2044	17.028	77.29	4.3476	28.010	89.35	4.4926	64.054	84.68	4.4389
8.000	45.46	3.8168	12.000	62.50	4.1352	21.000	72.92	4.2894	35.060	86.20	4.4567	80.005	82.54	4.4133
10.007	33.91	3.5237	15.139	55.60	4.0182	24.090	69.80	4.2457	47.028	81.52	4.4008	96.000	77.58	4.3513
			18.000	48.66	3.8848	30.005	63.21	4.1465	64.007	73.15	4.2925	115.00	74.95	4.3162
						34.000	58.81	4.0744	75.000	67.98	4.2192			
						38.028	54.84	4.0044	95.015	59.82	4.0913			
									115.00	50.85	3.9289			

*A/A₀ : The ratio of chloramphenicol content remained at any time

(A) to the initial chloramphenicol content (A₀).

a : average from three values (n = 3).

Table 5 The Statistic Values (r^2, k, F, sk) of Chloramphenicol
Which were Calculated* from Data Presented in Table 4.

Statistic Values / Temp. (°C)	70°	60°	50°	40°	33°
r^2	0.9796	0.9934	0.9994	0.9930	0.9836
F	287.68	1047.22	10327.3	1127.01	420.51
k (day^{-1})	1.02×10^{-1}	3.38×10^{-2}	1.37×10^{-2}	5.28×10^{-3}	2.60×10^{-3}
sk	6.02×10^{-3}	1.05×10^{-3}	1.57×10^{-4}	1.57×10^{-5}	1.27×10^{-5}

*Degradation of chloramphenicol is pseudo-first order (8).

:Use SPS program for calculation these statistic values.

Table 6 Data of Chloramphenicol:β-CD Complex at 70°, 60°, 50° 40°C and at Room Temperature (33°C).

70°C [a]			60°C [a]			50°C [a]			40°C [a]			Room Temp(30-35) [a]		
day	%A/A_o	ln A/A_o	day	%A/A_o	ln A/A_o	day	%A/A_o	ln A/A_o	day	%A/A_o	ln A/A_o	day	%A/A_o	ln A/A_o
0.000	93.77	4.5408	0.000	93.03	4.5329	0.000	98.51	4.5902	0.000	100.49	4.6101	0.000	99.25	4.5976
1.000	90.86	4.5093	1.000	91.65	4.5180	1.000	98.10	4.5860	1.000	100.25	4.6077	7.000	98.92	4.5943
2.005	87.56	4.4723	2.005	89.87	4.4984	4.005	97.05	4.5752	3.007	99.70	4.6022	14.026	96.83	4.5730
3.007	84.19	4.4331	3.007	88.74	4.4857	7.000	96.28	4.5673	9.007	99.20	4.5971	21.010	97.84	4.5833
4.054	81.24	4.3974	5.028	85.18	4.4448	10.007	94.98	4.5537	15.000	98.85	4.5936	35.007	96.90	4.5737
5.028	78.40	4.3618	7.000	83.12	4.4203	12.976	93.82	4.5414	21.000	97.56	4.5805	50.000	96.10	4.5654
6.064	75.20	4.3202	10.007	78.80	4.3669	17.028	92.29	4.5249	28.010	96.42	4.5687	64.054	94.38	4.5473
8.000	68.43	4.2258	12.000	75.80	4.3281	21.000	90.94	4.5102	35.060	95.60	4.5602	80.005	94.02	4.5435
10.007	62.59	4.1366	15.139	70.96	4.2621	24.090	90.73	4.5079	47.028	94.06	4.5439	96.000	91.97	4.5215
			18.000	67.18	4.2074	30.005	87.85	4.4756	64.007	91.62	4.5176	115.00	92.05	4.5223
						34.000	86.26	4.4574	75.000	89.92	4.4989			
						38.028	84.53	4.4371	95.015	87.23	4.4685			
									115.00	83.97	4.4304			

A/A_o : The ratio of the complex content remained at any time (A) to the initial complex content (A_o).

a : average from three values (n = 3).

<u>Table 7</u>. The Statistic Values (r^2, k, F, sk) of Chloramphenicol:

β-CD complex Which were Calculated from Data Presented

in Table 6.

Statistic Values / Temp. (°C)	70°	60°	50°	40°	33°
r^2	0.9959	0.9967	0.9925	0.9957	0.9289
F	1449.82	1808.83	923.62	1859.95	78.36
k (day^{-1})	4.13×10^{-2}	1.77×10^{-2}	3.73×10^{-3}	1.45×10^{-3}	7.105×10^{-5}
sk	1.08×10^{-3}	4.15×10^{-4}	1.23×10^{-4}	3.37×10^{-5}	8.03×10^{-5}

:Use SPS program for calculation these statistic values.

*Degradation of chloramphenicol is pseudo-first order (8).

Table 8 Arrhenius Relationship of Chloramphenicol base.

°C	Temp. $\frac{1}{T} \times 10^3 (°K^{-1})$	Specific rate constant of Chloramphenicol, (day^{-1})		
		$k \pm sk \times 10^4$	ln k	$\ln(k+sk) - \ln(k-sk)$
70	2.9155	1020.93 ± 60.2	-2.2819	-2.2246 to -2.3426
60	3.0030	338.56 ± 10.5	-3.3858	-3.3551 to -3.4171
50	3.0960	137.24 ± 1.35	-4.2889	-4.2788 to -4.2985
40	3.1949	52.75 ± 1.57	-5.2457	-5.2154 to -5.2750
Arrh. equation	$\ln k = 28.242 - 10497.61 (\frac{1}{T})$			
r^2	0.9957			
F	460.68			
ΔH_a (cal/mol)	20858.75 (20.858 kcal/mol)			
Extra. Value at 33°C	$33°C = 3.2680 \times 10^{-3} (°K^{-1})$			
$\hat{k} (day^{-1})$	2.3246×10^{-3}			
$\hat{k} \pm s\hat{k}$	2.2370×10^{-3} to 2.4231×10^{-3}			

Table 9   Arrhenius Relationship of Chloramphenicol:β-CD Complex.

°C	Temp. $\frac{1}{T} \times 10^3 (°K^{-1})$	Specific rate constant of Complex		
		$k \pm sk \times 10^4$	$\ln k$	$\ln(k \pm sk) - \ln(k \pm sk)$
70	2.9155	412.69 ± 10.8	−3.1876	−3.1618 to −3.2142
60	3.0030	176.69 ± 4.15	−4.0359	−4.0127 to −4.0597
50	3.0960	37.34 ± 1.23	−5.5913	−5.5579 to −5.6238
40	3.1949	14.57 ± 0.337	−6.5307	−6.5085 to −6.5548
Arrh. equation	$\ln k = 33.112 - 12432.63(\frac{1}{T})$			
r^2	0.9869			
F	150.867			
ΔHa (cal / mol)	24703.64 (24.70 kcal / mol)			
Extra. Value at 33 °C	$33°C = 3.2680 \times 10^{-3} (°K^{-1})$			
$\hat{k}$ (day^{-1})	5.4331×10^{-4}			
$\hat{k} \pm s\hat{k}$	5.0021×10^{-4} to 5.9018×10^{-4}			

Table 10 The Extrapolated Shelf-life Values and the Apparent
Shelf-life Values of Chloramphenicol and Chloramphenicol:
β-CD Complex at Room Temperature (33°C).

	Extrapolated Values (months)	Apparent Values (months)
Chloramphenicol	2.63-3.14	2.35-2.80
complex	10.8-14.0	8.04-11.2

Table 11   Antimicrobial Activity Test of Chloramphenicol vs

Chloramphenicol:β-CD Complex.

DHS	Zone diameter of Various Standard (mm)[a]								Zone diameter of sample and Standard (mm)[a]			
Conc (µg/ml) / Plate No.	32.0	50.0	40.0	50.0	62.5	50.0	78.1	50.0	Chloram 50.0	Std 50.0	Complex 50.0	Std 50.0
1	17.38	19.98	19.20	20.68	21.30	20.16	22.06	20.86	19.92	19.76	20.24	20.32
	17.64	20.24	18.92	20.22	21.28	20.52	22.08	20.20	20.08	20.54	20.26	20.74
	18.04	20.72	19.50	20.56	21.18	20.56	22.28	20.24	19.92	19.94	20.84	20.28
2	17.80	20.36	19.21	20.20	20.88	19.98	22.78	19.98	20.48	18.98	20.24	20.08
	18.20	20.08	19.10	20.38	21.32	20.30	22.58	20.38	19.50	20.24	20.96	20.58
	17.50	20.06	19.22	20.50	21.28	20.30	22.64	20.92	19.06	19.52	19.46	20.58
3	17.16	20.02	19.08	20.96	21.40	19.72	22.72	20.10	19.98	20.02	19.58	20.26
	17.64	20.02	19.26	20.92	21.14	20.80	23.42	20.70	20.06	20.00	20.38	20.38
	17.68	20.70	19.25	20.90	21.02	20.86	22.56	20.68	20.06	20.22	20.30	20.82
Sum of g readings	159.04	182.34	172.74	185.24	190.88	183.2	203.12	184.06	179.06	180.22	182.34	184.04
Average of g readings	17.67	20.26	19.19	20.58	21.21	20.36	22.57	20.45	19.90	20.02	20.26	20.45
Correction zone diameter of 50ug/ml	+ 0.15		- 0.17		+ 0.05		- 0.04		+ 0.39		- 0.04	
corrected value	17.82		19.02		21.26		22.53		20.29		20.22	

a : average from three values (n = 3).
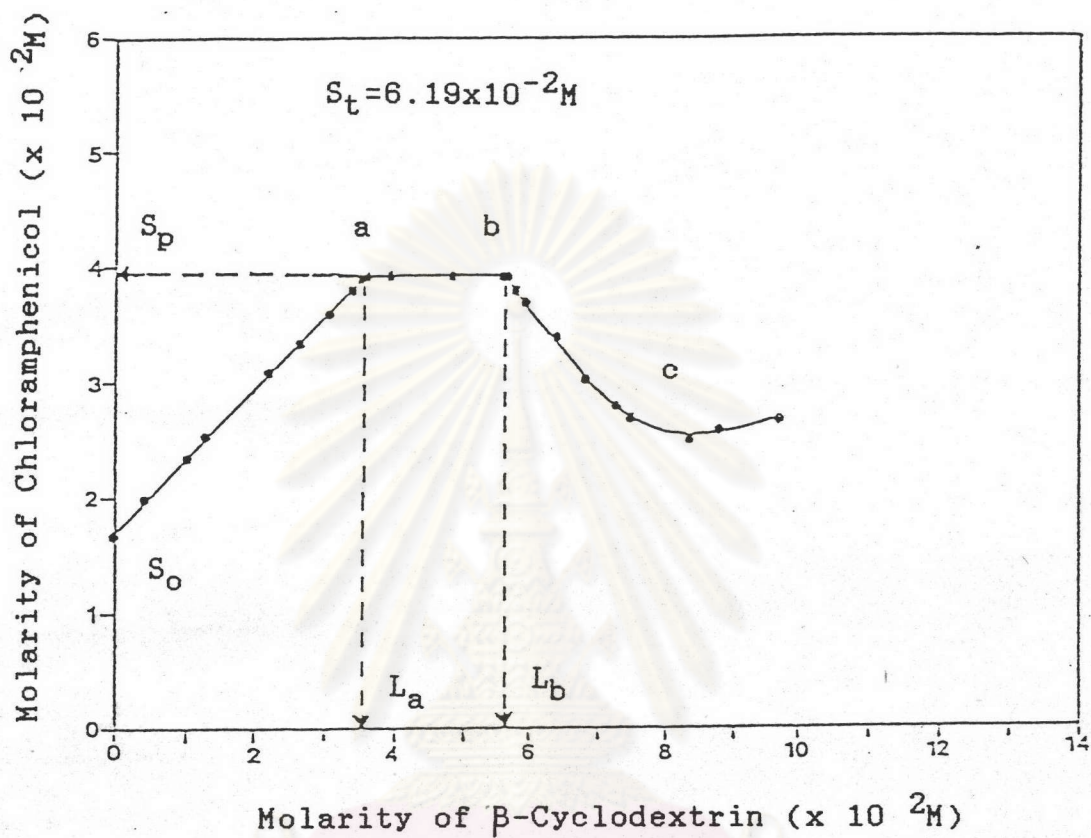
Figure 4 Phase Solubility Diagram of Chloramphenicol:
B-Cyclodextrin in Water at Room Temperature.
Stoichiometric Ratio = 1:1
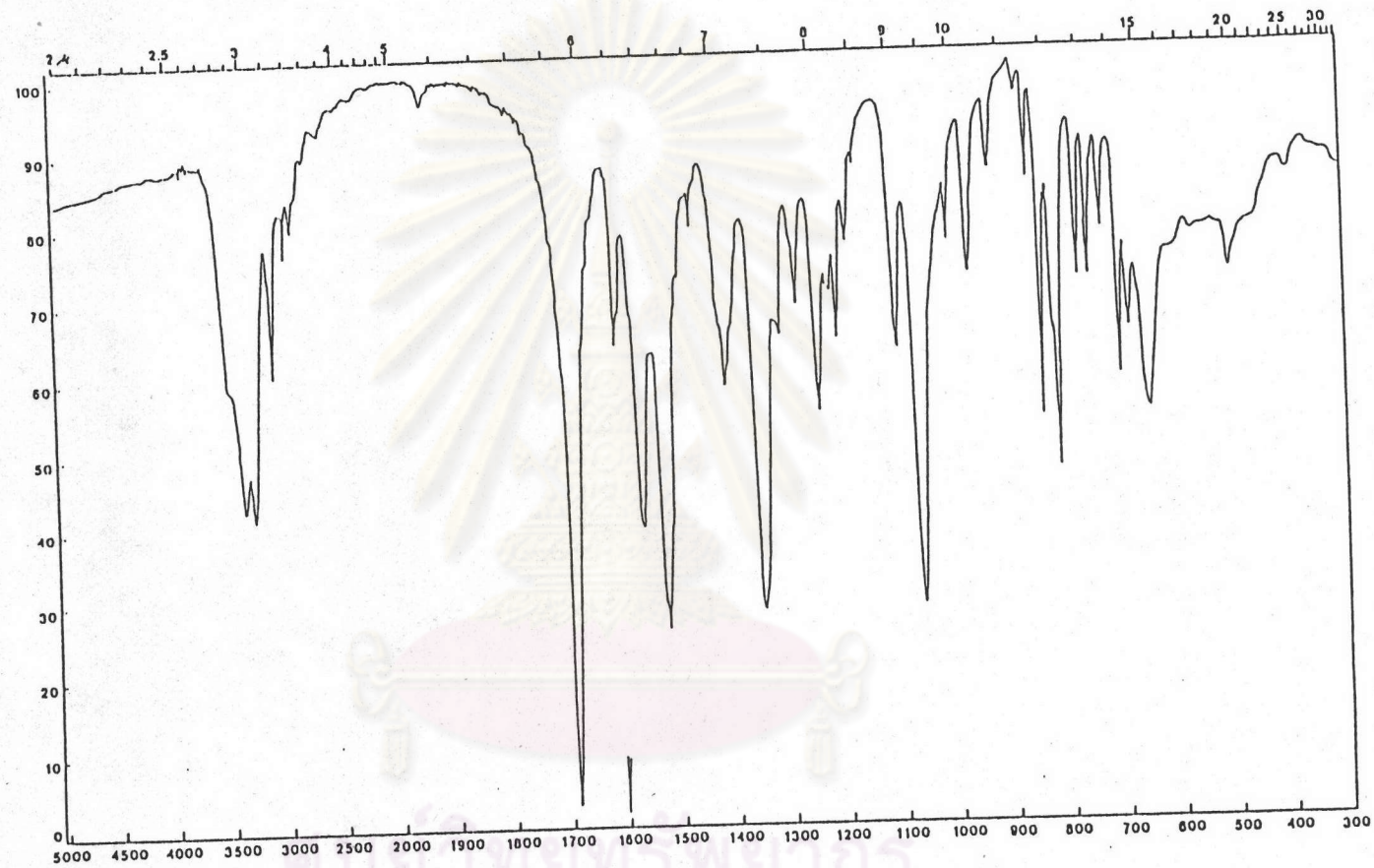
Figure 5   IR spectrum of Chloramphenicol base.
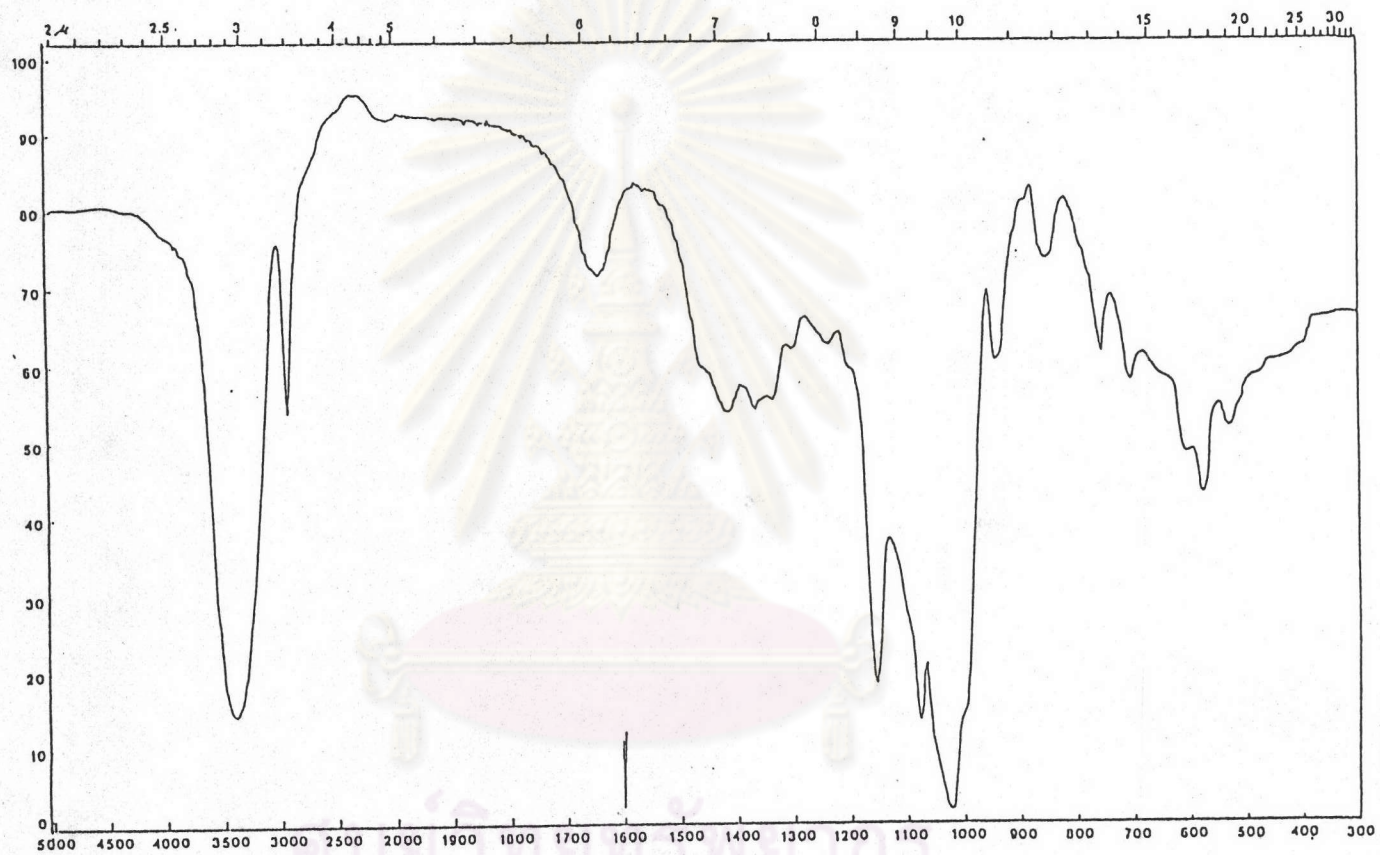
Figure 6   IR spectrum of β-Cyclodextrin.
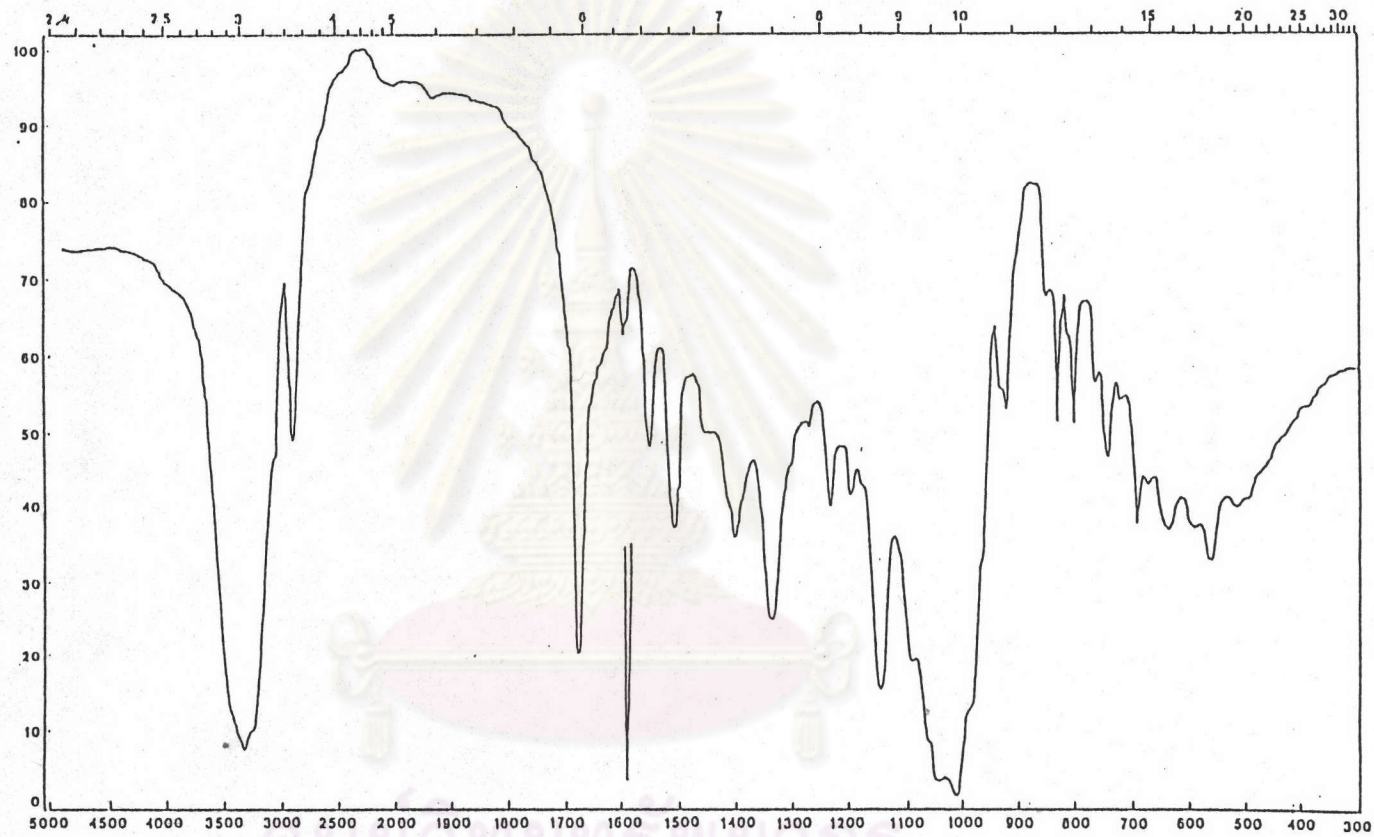
Figure 7 IR spectrum of Physical Mixture 1:1 of
Chloramphenicol:β-Cyclodextrin.
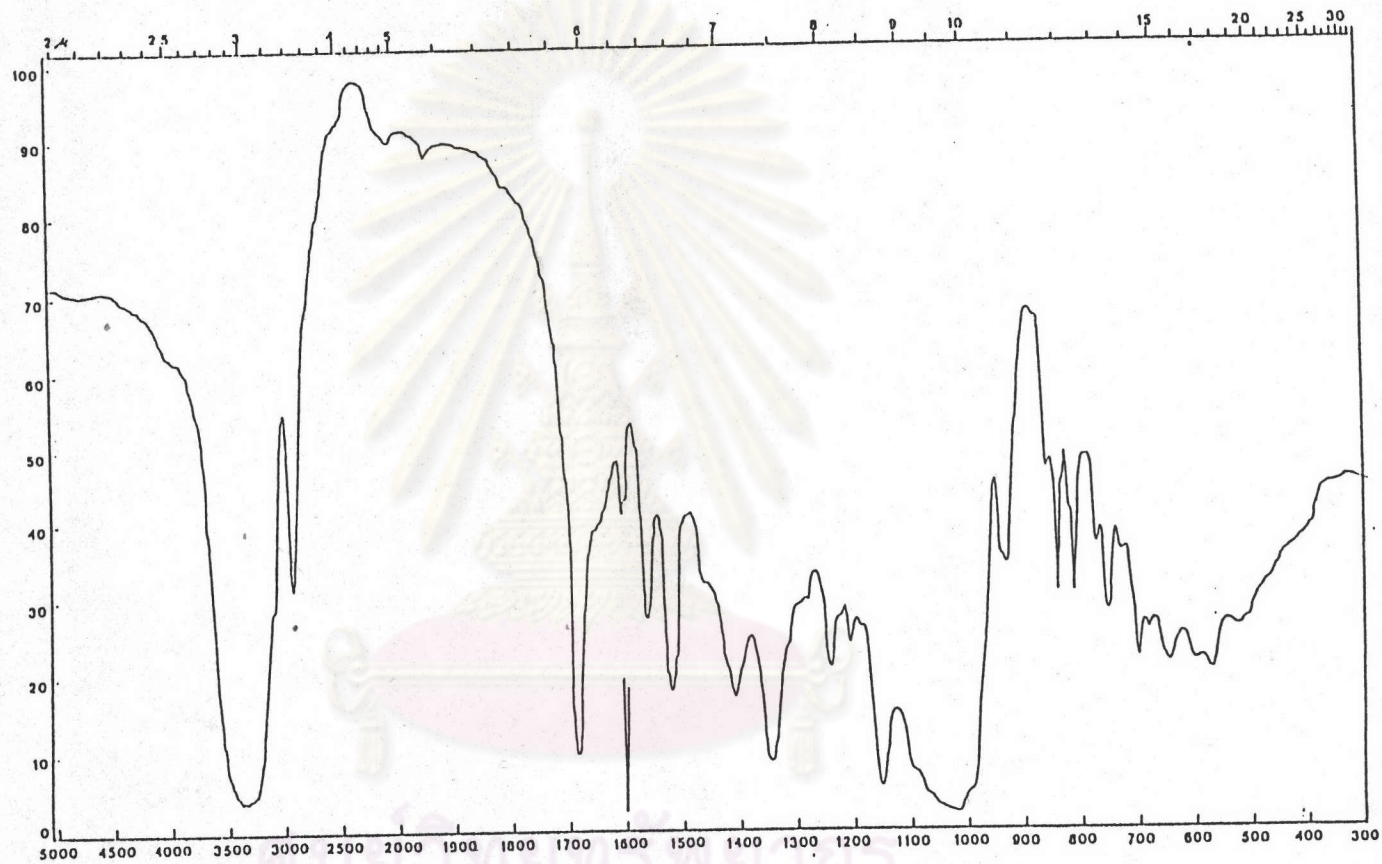
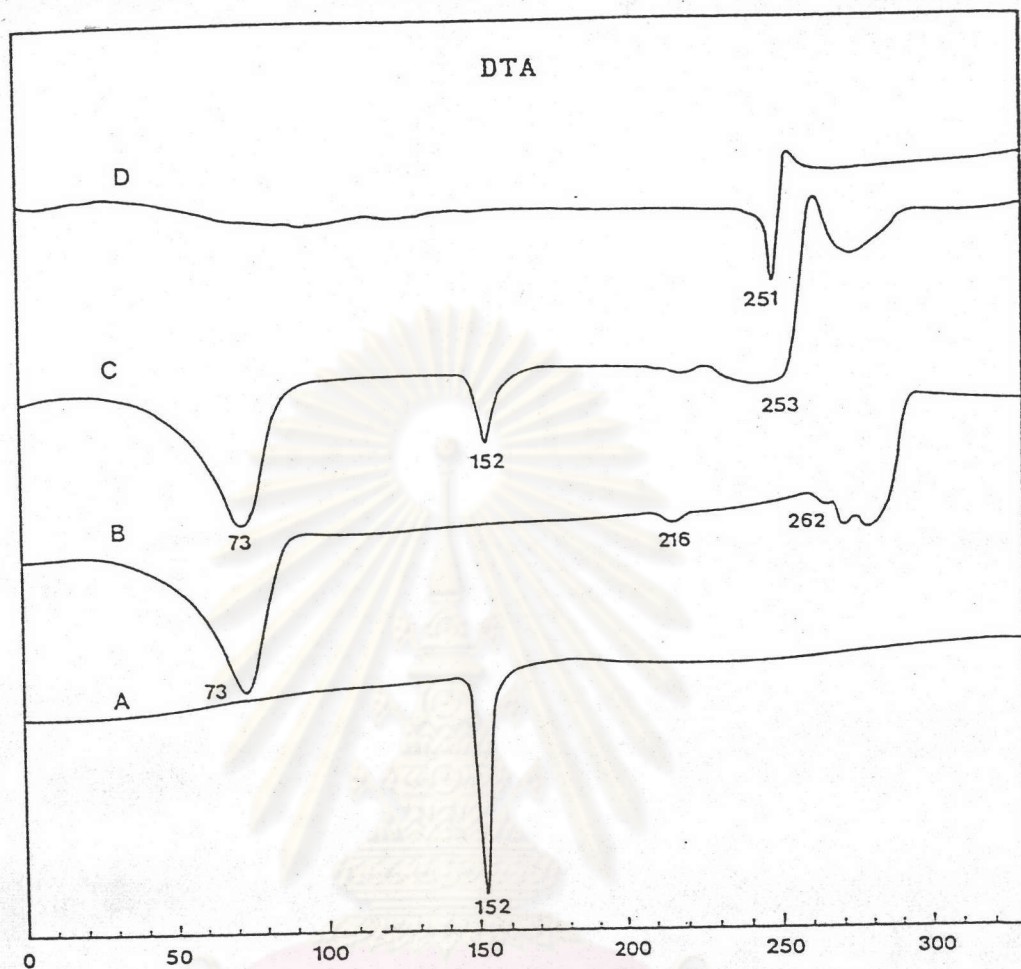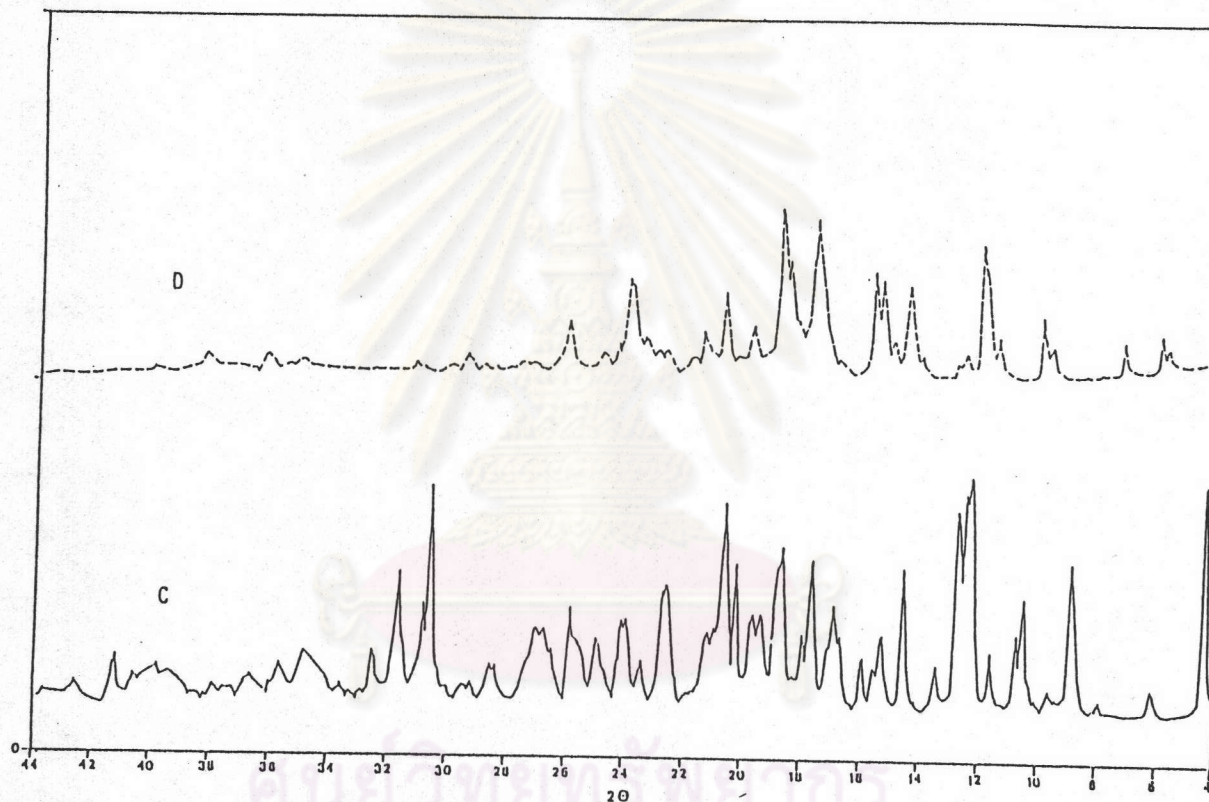Figure 8 IR spectrum of Chloramphenicol:β-Cyclodextrin Complex.

Figure 9 Differential Thermal Analysis (DTA).

A: Chloramphenicol

B: β-Cyclodextrin

C: Physical Mixture 1:1

D: Chloramphenicol:β-Cyclodextrin Complex

Figure 10 X-ray Diffraction Patterns.

C: Physical Mixture 1:1

D: Chloramphenicol:β-Cyclodextrin Complex
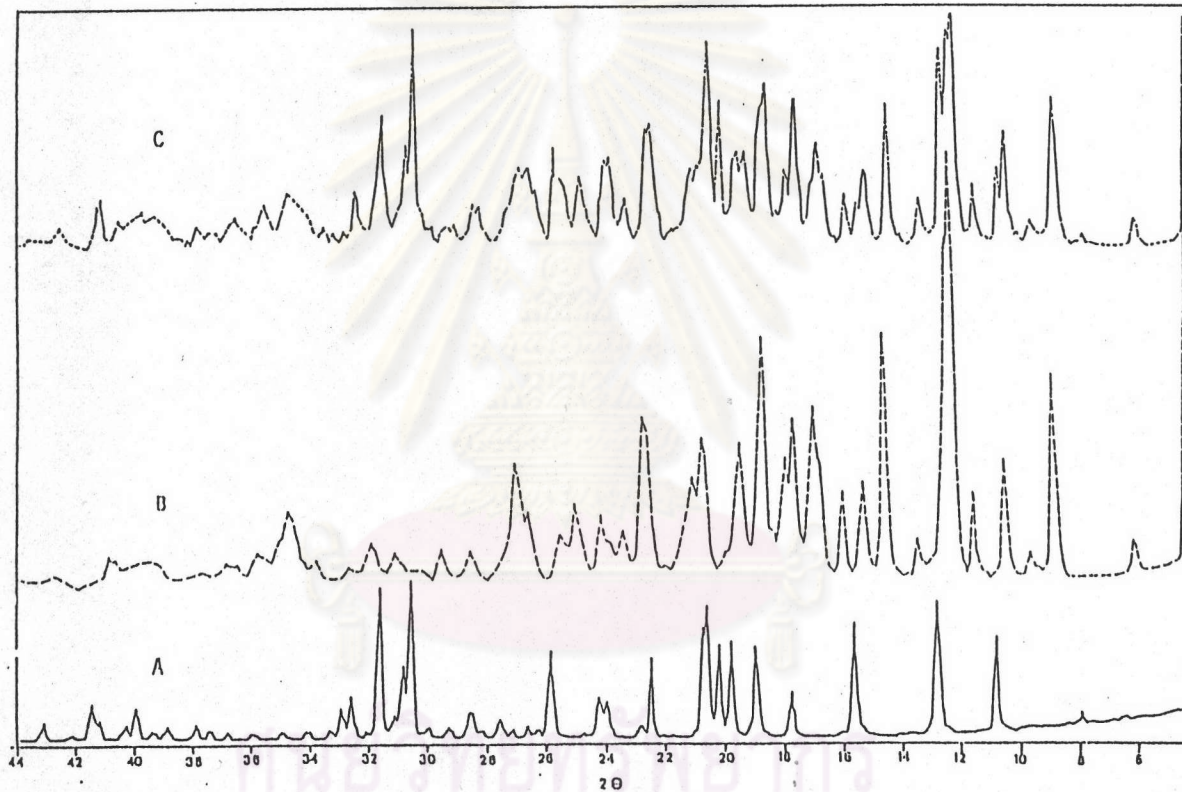
Figure 11 X-ray Diffraction Pattern of Chloramphenicol (A),
β-Cyclodextrin (B), Physical Mixture (C).
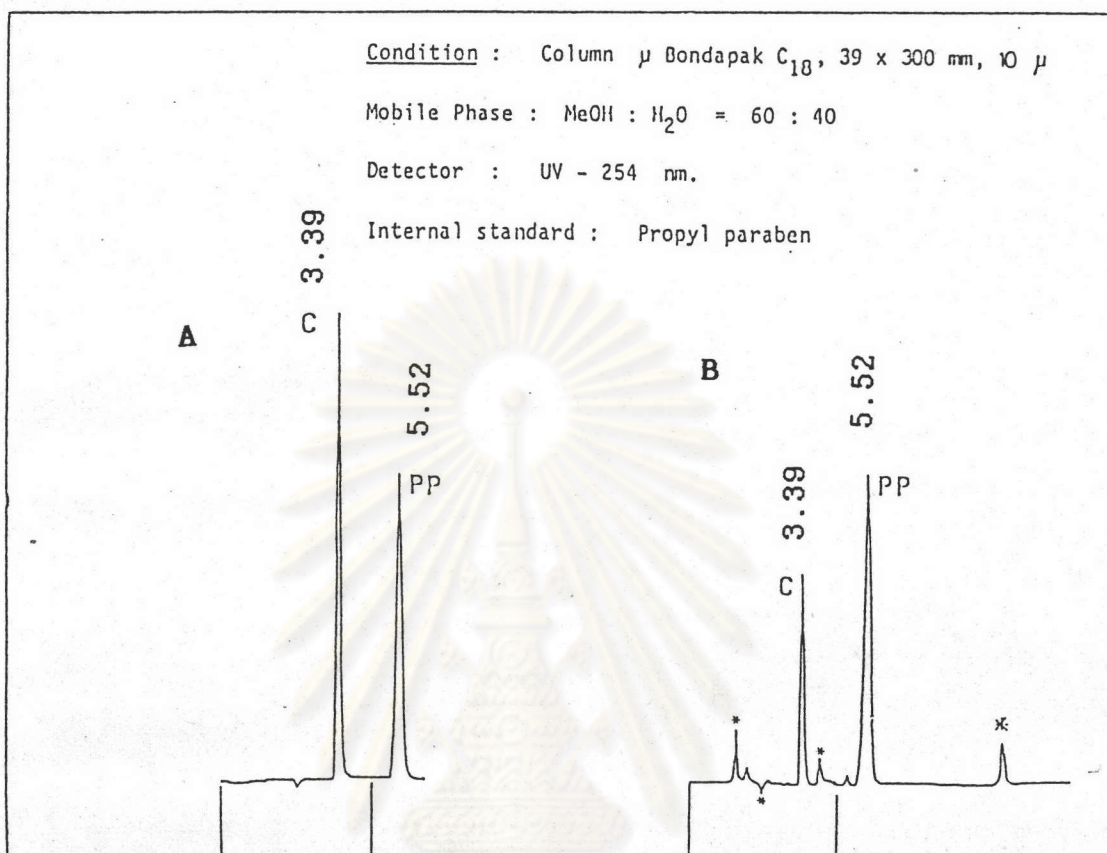
Figure 12 Chromatogram by HPLC of Chloramphenicol.

A: Chromatogram before degradation.

B: Chromatogram after degradation.

C = Chloramphenicol

PP = Propyl paraben

Column: u bondapak C_{18}, 39 x 300 mm, 10 u

Mobile Phase: MeOH:H_2O = 60:40

Detector: UV 254 nm

Injection Volume: 20 ul

Internal Standard: Propyl paraben
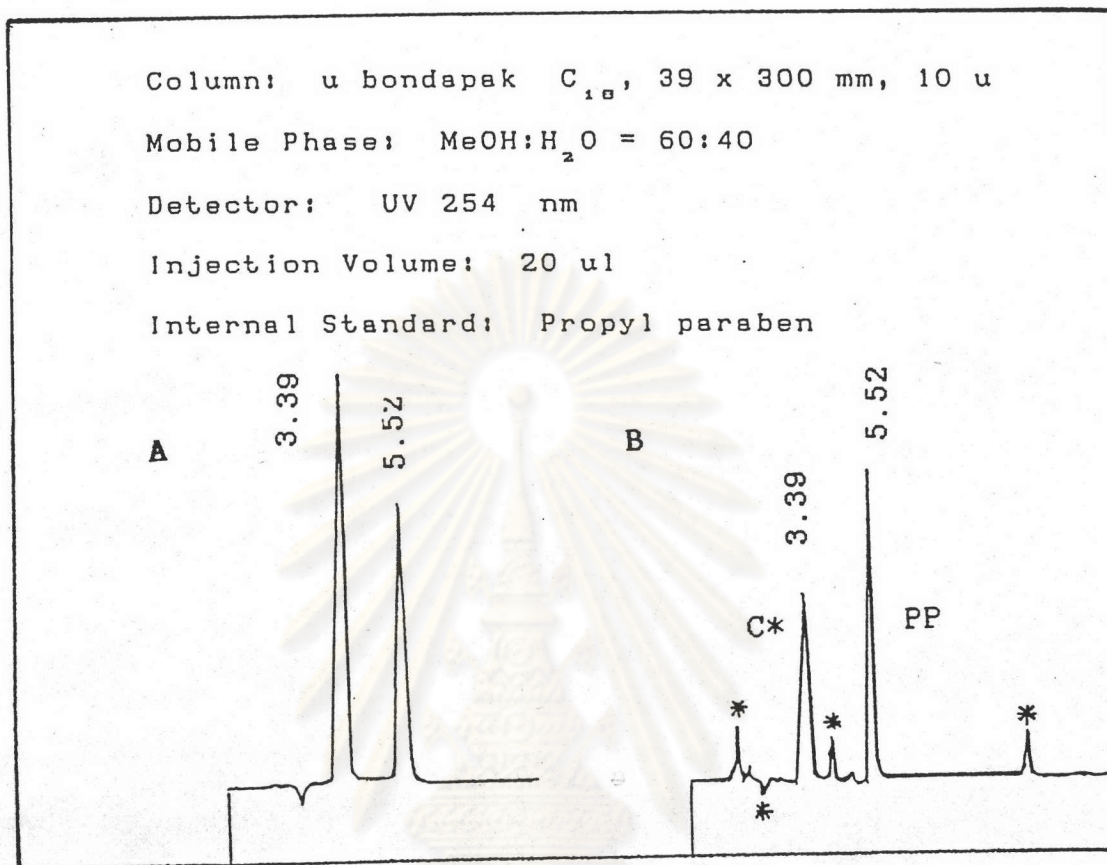
Figure 13 Chromatogram by HPLC of Chloramphenicol:

β-Cyclodextrin Complex.

C* = Chloramphenicol:β-Cyclodextrin Complex.

PP = Propyl paraben.

A = Chromatogram before degradation.

B = Chromatogram after degradation.
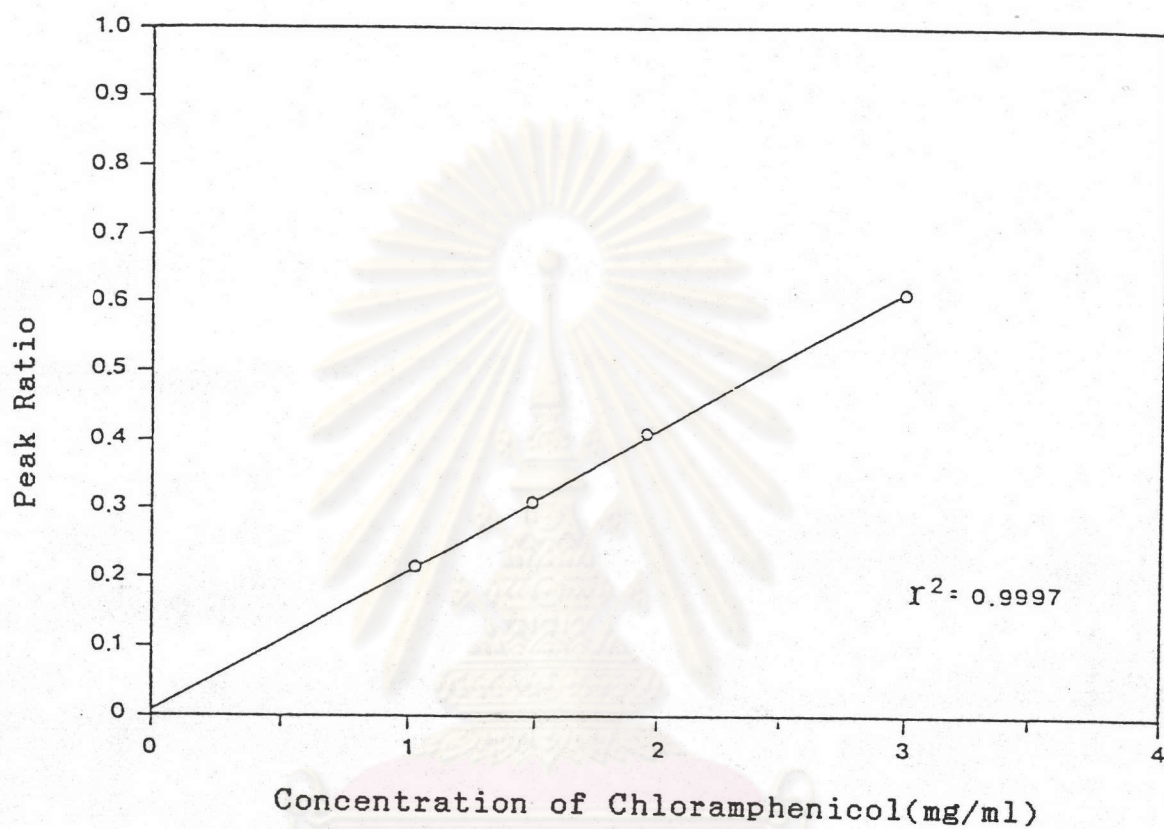
Figure 14   Standard Curve of Chloramphenicol.
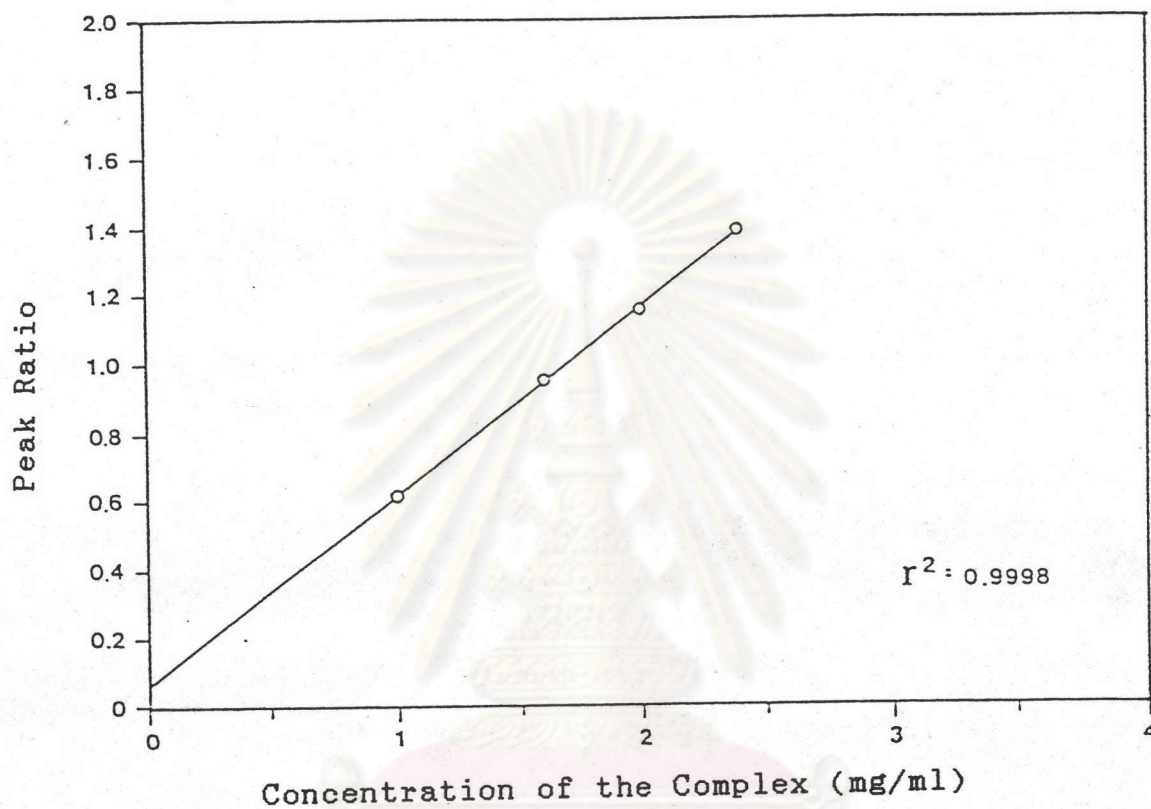
Figure 15 Standard Curve of Chloramphenicol:

β-Cyclodextrin Complex.
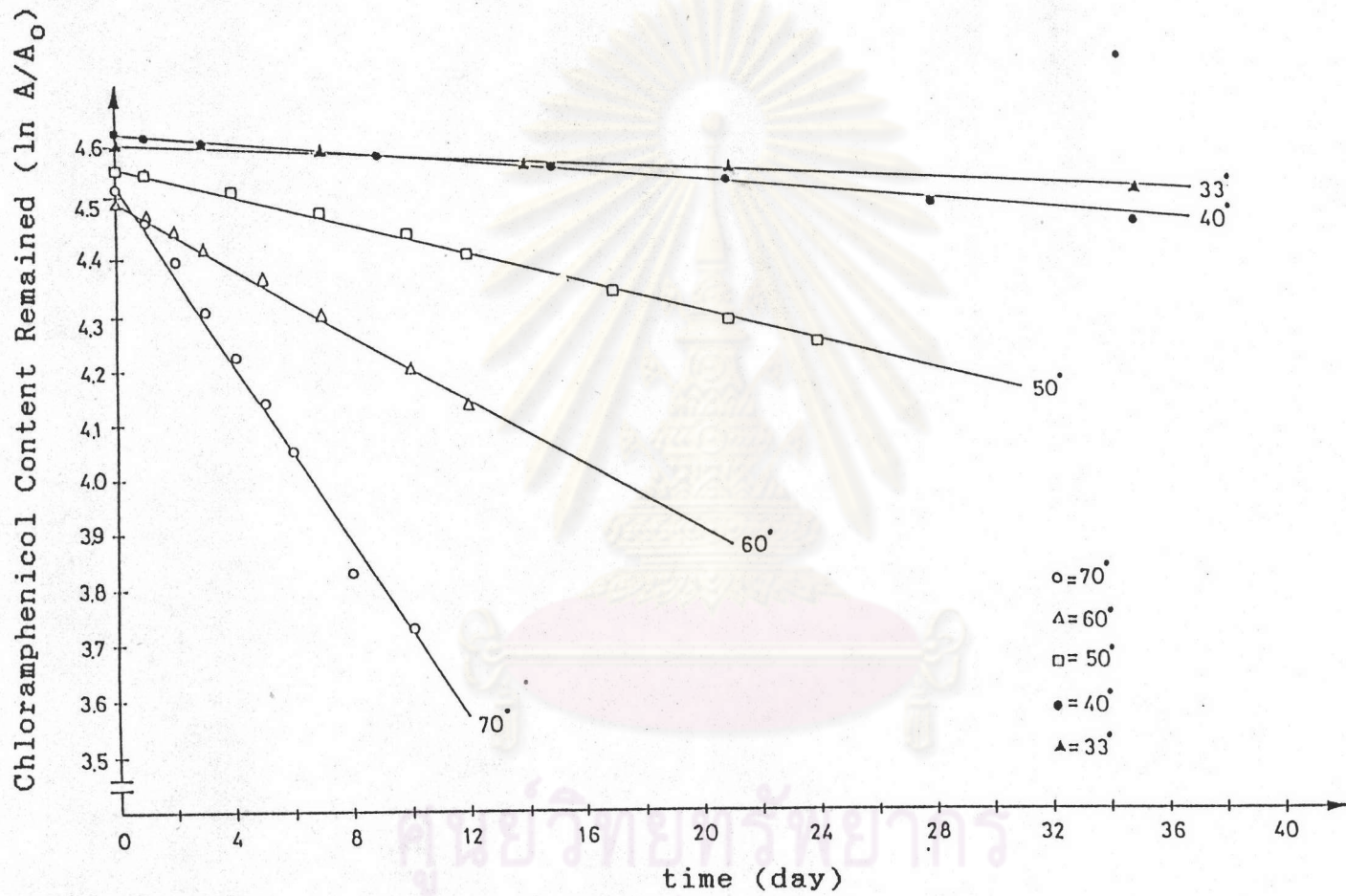
Figure 16 Degradation Rate Constant of Chloramphenicol at Various Temperature.
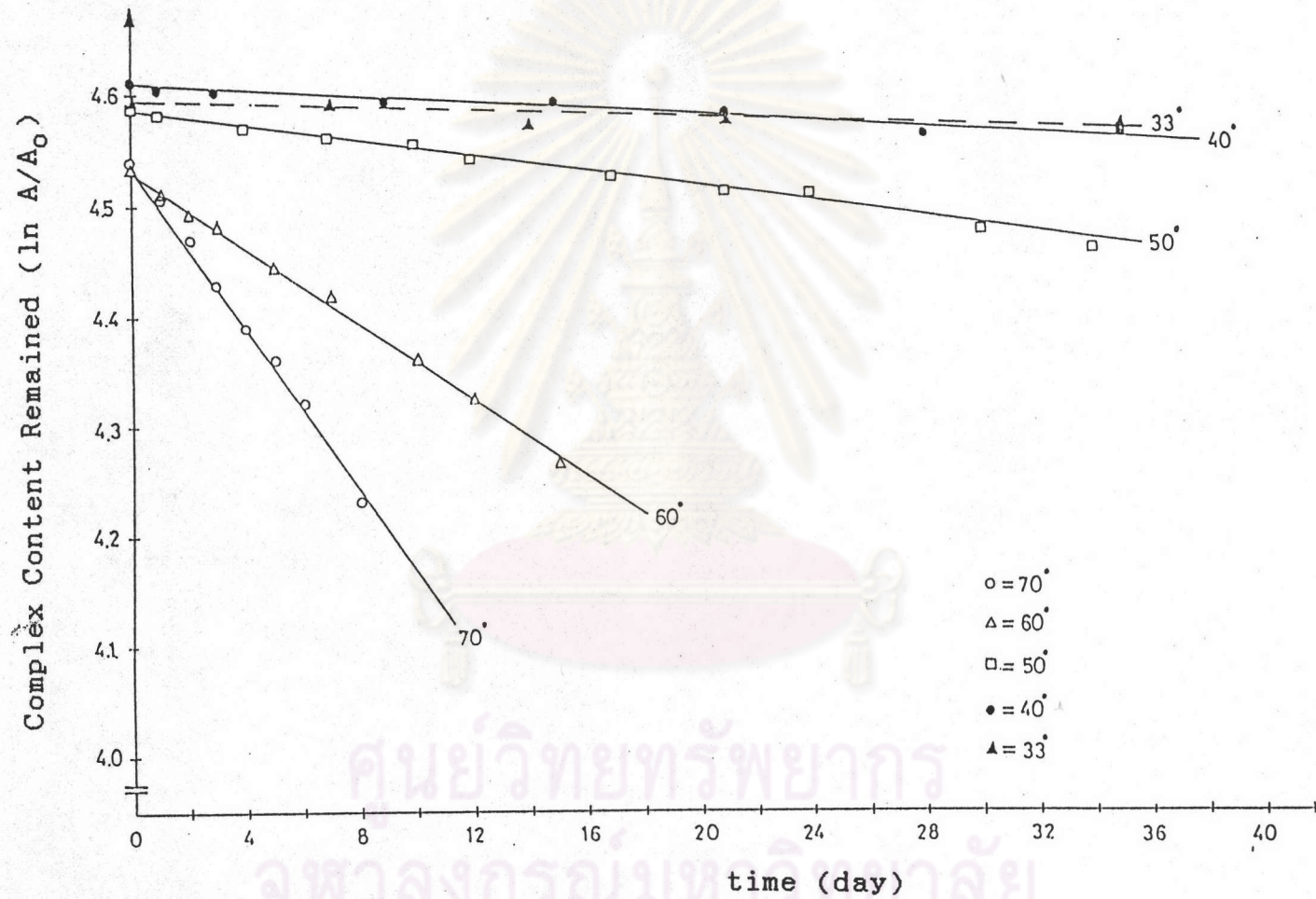
Figure 17   Degradation Rate Constant of Chloramphenicol:
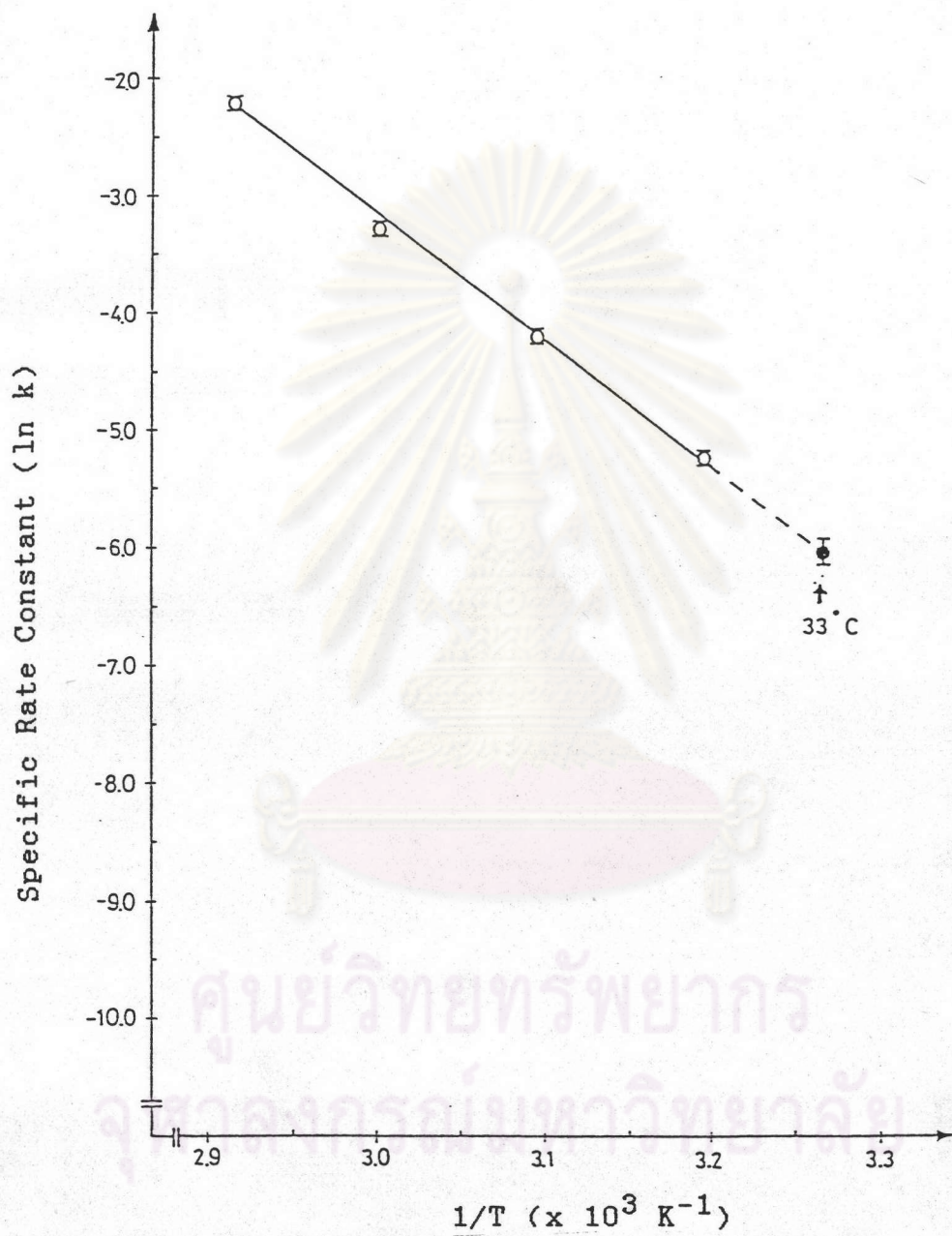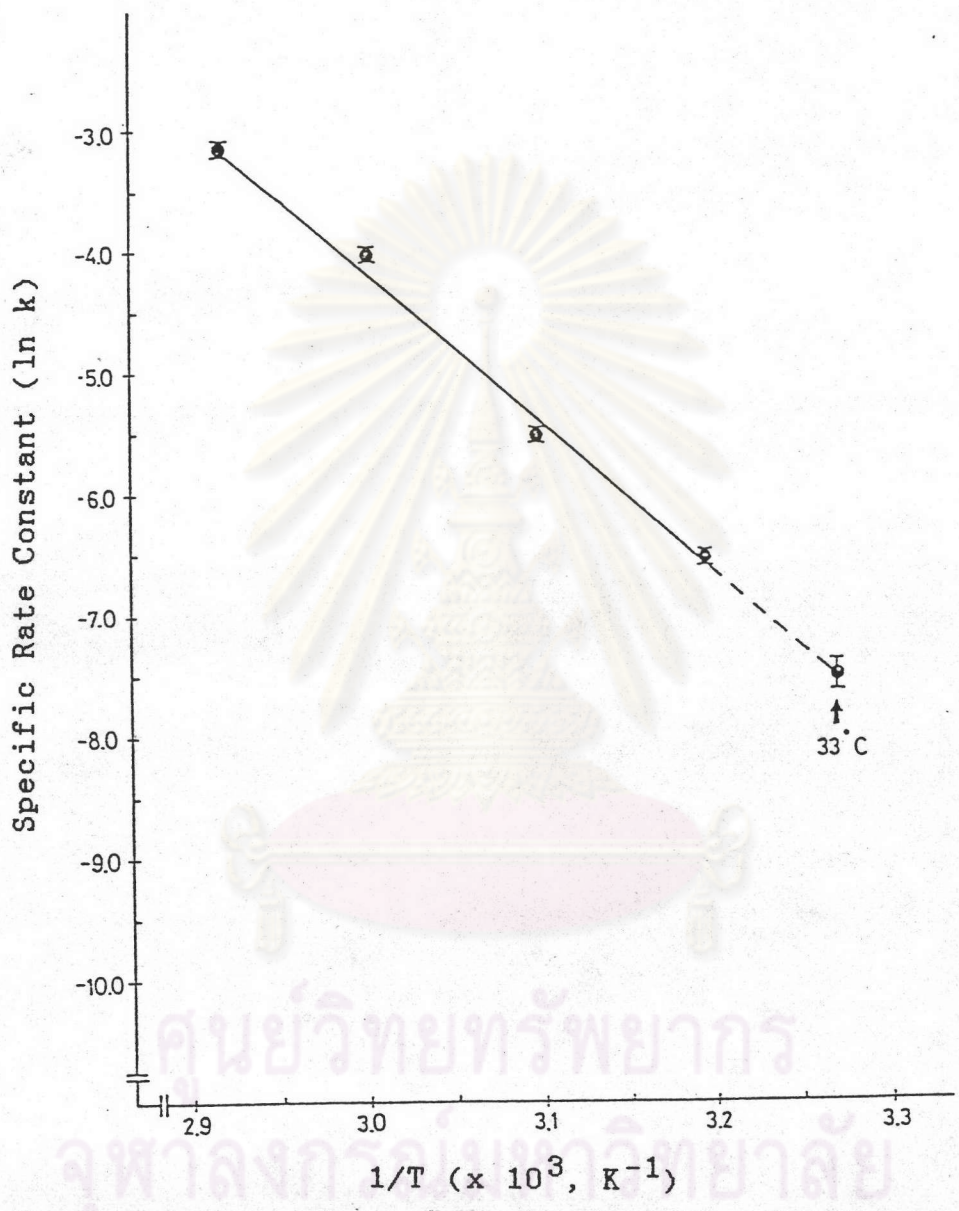β-Cyclodextrin at Various Temperature.

Figure 18 Arrhenius Plot of Chloramphenicol.

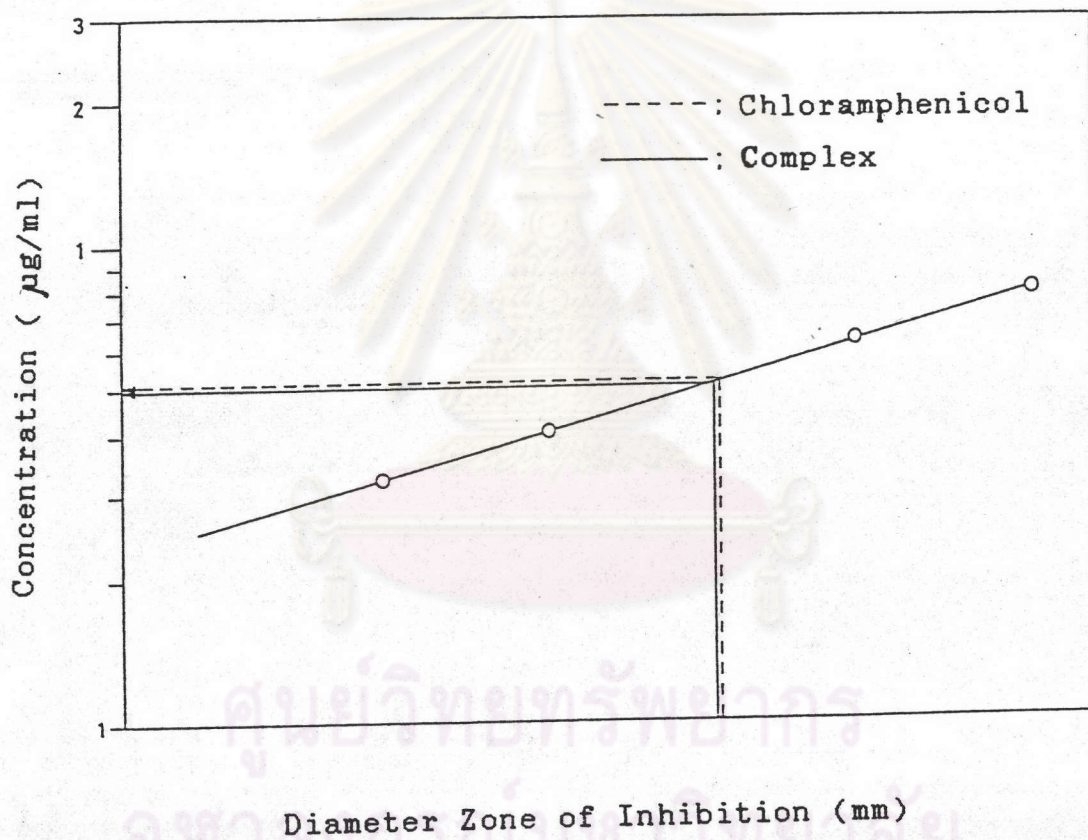Figure 19 Arrhenius Plot of Chloramphenicol: β-Cyclodextrin Complex.

Figure 20   Microbiological Assay.

Title: Chloramphenicol,Complex.   Substrate: Antibiotic Medium I.

Organism: *Micrococcus leuteus*(ATTC 9341)

Inoculation Intensity: (Approximately Test)   Initial pH: 6.5-6.6.

VITA

Miss Anong Patmasiriwat was graduated from the faculty of Pharmacy, Chiengmai University in 1984. Then, she worked as an instructor in department of Pharmaceutics, at the faculty of pharmaceutical science, Prince of Songkla University from 1984-1985. And, she has been worked as an instructor in department of Pharmaceutical Chemistry at the faculty of Pharmacy, Rungsit College since october, 1989.