CHAPTER III

RESULTS

Determination of Maximum Absorption Wavelength of Chloramphenicol, Chloramphenicol : 2-HP-β-CD and 2-HP-β-CD

In the preliminary study, UV scanning for maximum absorption wavelength of chloramphenicol and chloramphenicol : 2-HP-β-CD at the same concentration (25 μg/ml) were shown in Figure 9. Their maxima UV-absorption were detected at the same wavelength of 279 nm. The extinction coefficients (E1%, 1cm) of chloramphenicol and chloramphenicol : 2-HP-β-CD were 272.8 and 270.8, respectively. Moreover, 2-HP-β-CD did not absorped at wavelength 279 nm. Thus, this determination indicated that 2-HP-β-CD did not interfere with the spectrophotometric measurements and the quantitative analysis of chloramphenicol in phase solubility study by UV absorption was performed at the wavelength of 279 nm.

Phase Solubility Analysis

Figure 11 showed the phase solubility diagram of chloramphenicol in aqueous solubility of 2-HP-β-CD at 27±1 °C. Phase solubility diagram was constructed by plotting the molarity of chloramphenicol found in solution against the molarity of 2-HP-β-CD added. Phase solubility data between chloramphenicol and 2-HP-β-CD were shown in Table 7. From the phase solubility diagram, the solubility of chloramphenicol increased linearly with increasing the 2-HP-β-CD concentration. The data indicated that an A type relationship as described by Higuchi and Conner (1965) was produced. In the absence of 2-HP-β-CD, the solubility of chloramphenicol in water at 27±1°C was 1.38×10^{-2} M. From the slope of the phase solubility curve having a value of 0.62, it indicated that the formation of the complex might occur in stoichiometric ratio of both 1 : 1 and 1 : 2 type. Since it was difficult to determine the concentration of each complex, the stability constant (the formation constant) k_C of the complex at low 2-HP-β-CD concentration (< 19 % w/v) (Loftsson et al, 1989) was calculated based on the assumption of 1 : 1 complex according to the equation;

$$k_C = \frac{\text{slope}}{\text{intercept (1-slope)}}$$

was found to be 118 M^{-1}.

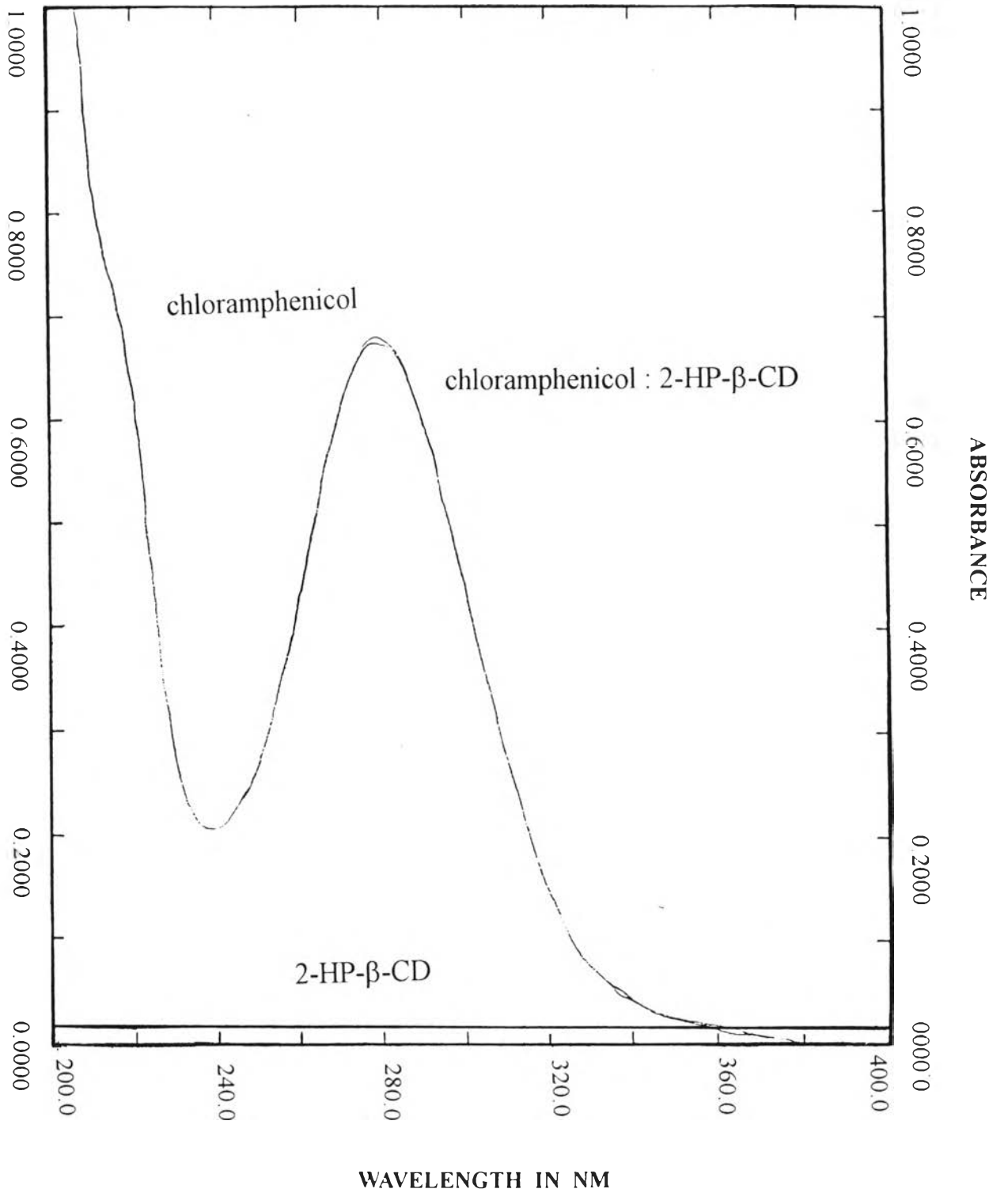

Figure 9 UV Scanning for maximum absorbance wavelength of chloramphenicol, chloramphenicol : 2-HP-β-CD and 2-HP-β-CD.

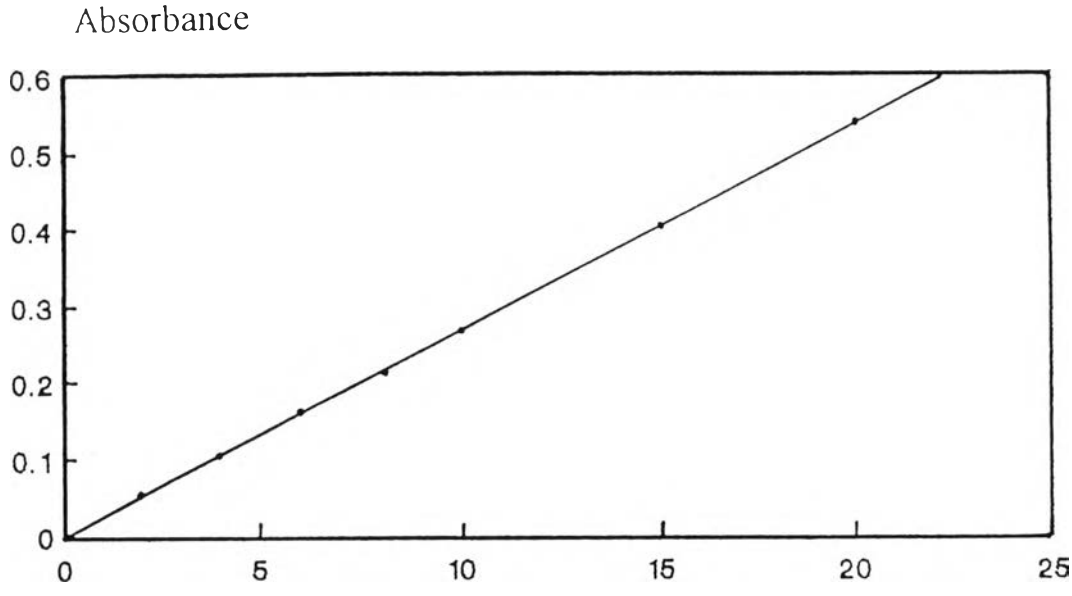

Figure 10 Standard curve of chloramphenicol (UV spectrophotometry).

Table 7 Phase solubility data of chloramphenicol : 2-HP-β-CD in water at room temperature.

Molarity of 2-HP-β-CD $x10^2$ M	Molarity of chloramphenicol $x10^2$ M
0	1.38
0.4	1.55
1.0	1.89
1.6	2.28
2.0	2.49
3.0	3.03
4.0	3.74
5.0	4.24
6.0	4.57
7.0	5.57
8.0	6.22
10.0	7.55
12.0	8.30
14.0	8.52

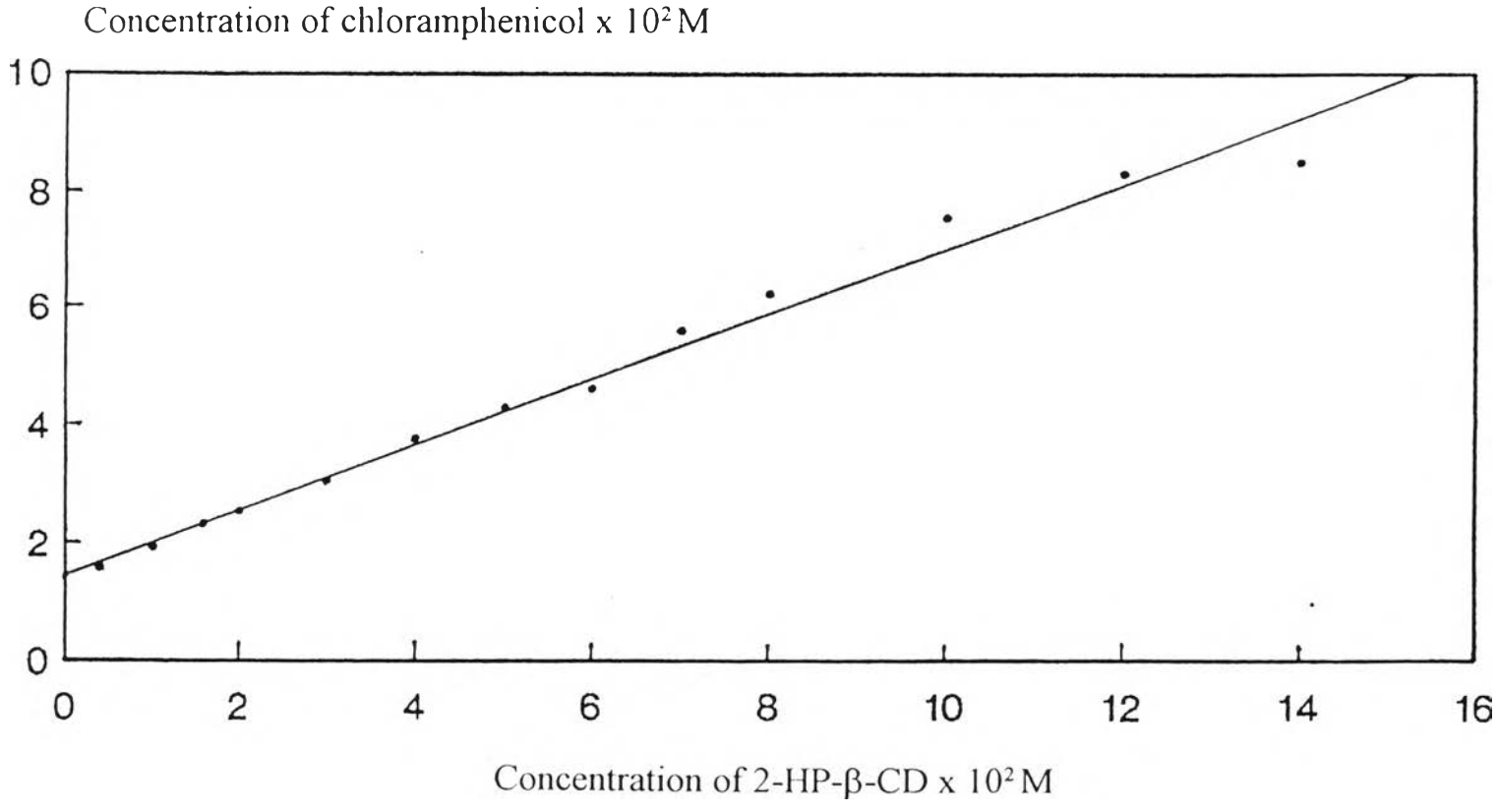

Figure 11 Phase solubility diagram of chloramphenicol : 2-HP-β-CD in water at room temperature.

Solid Chloramphenical : 2-HP-β-CD Complex Characteristics

Infrared spectrophotometry was employed in this study to verify the complex formation. The IR spectra of the pure drug, 2-HP-β-CD, physical mixture and freeze-dried product were compared in Figure 12.

The IR spectra of chloramphenicol showed the major peaks at 1686, 1564, 1521 and 1348 cm^{-1}, indicated C=O stretching of amide I, N-H bending of amide II, asymmetrically and symmetrically of N-O stretching ($ArNO_2$) from chloramphenicol molecule, respectively. It was found that N-H bending of amide II in solid complex was absent. In addition, the streching vibration peak of carbonyl group (C=O) in the complex was broaden than that from the physical mixture and the wave number was shift from 1686 cm^{-1} in the pure drug to 1692 cm^{-1} in the solid complex.

Thermograms of pure drug, 2-HP-β-CD, physical mixture and solid complex are compared in Figure 13. The themogram of pure drug gave a shape endothermic peak at 150 °C, whereas 2-HP-β-CD had no peaks around that temperature and found that 2-HP-β-CD showed the broad melting endothermic peak at 66° and 226 °C.The thermogram of physical mixture showed melting peak at 66°, 145° and 240 °C. In the case of solid complex, the thermogram showed no endothermic melting peak of chloramphenicol at 150 °C and of 2-HP-β-CD at 66 and 226 °C but showed the melting peak at 164 °C.

Figure 14 showed the X-ray diffractograms of the pure drug, 2-HP-β-CD, physical mixture and solid complex. 2-HP-β-CD gave a diffused diffraction pattern due to its amorphous character, which was contrast to the case of chloramphenicol which had crystalline pattern.The peak pattern of physical mixture was actually resulted from the combined peak patterns of chloramphenicol and 2-HP-β-CD. In the case of solid complex, the diffraction peaks of chloramphenicol disappeared and the pattern was apparently different from that of the physical mixture and giving an amorphous solid complex.

Determination of Physical Properties

1. Viscosity measurement

Viscosity at room temperature of various preparations was measured throughout 4 months and the values of mean were shown in Table 8.

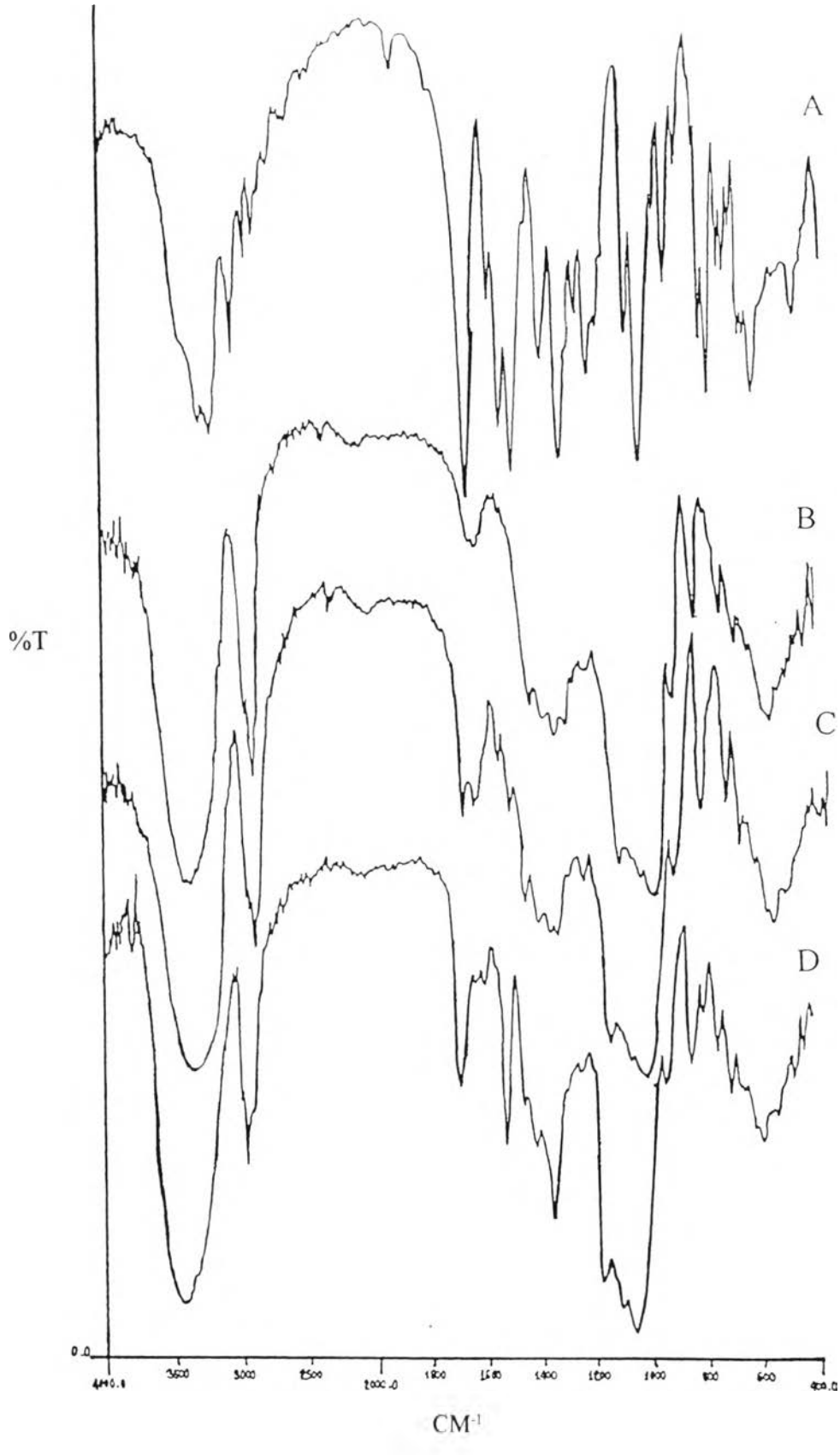

Figure 12 Comparison of FTIR spectrum.
A = Chloramphenicol
B = 2-HP-β-CD
C = Physical mixture (1 : 2)
D = Chloramphenicol : 2-HP-β-CD complex

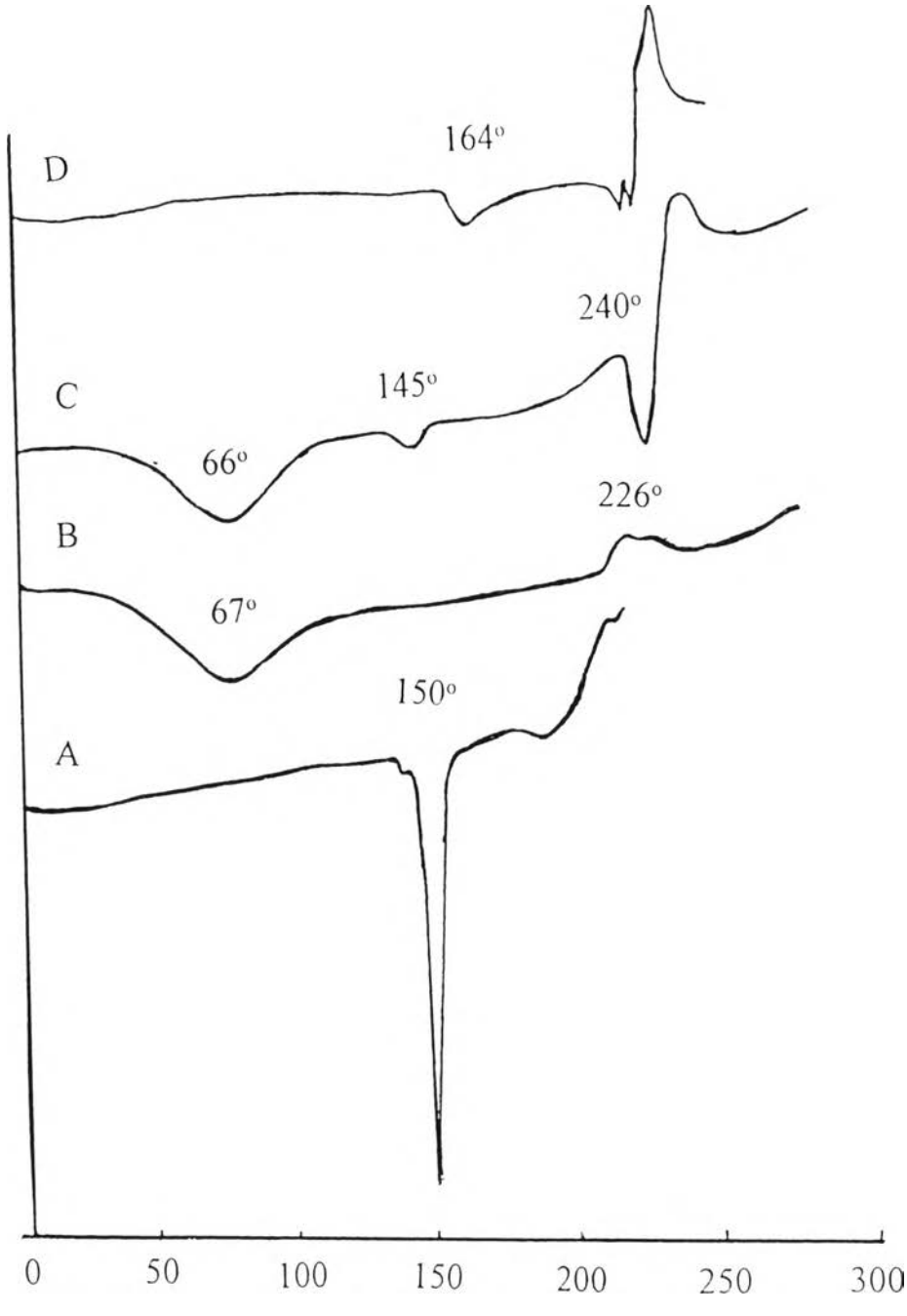

Figure 13 Differential thermal analysis (DTA).
A = Chloramphenicol
B = 2-HP-β-CD
C = Physical mixture (1 : 2)
D = Chloramphenicol : 2-HP-β-CD complex

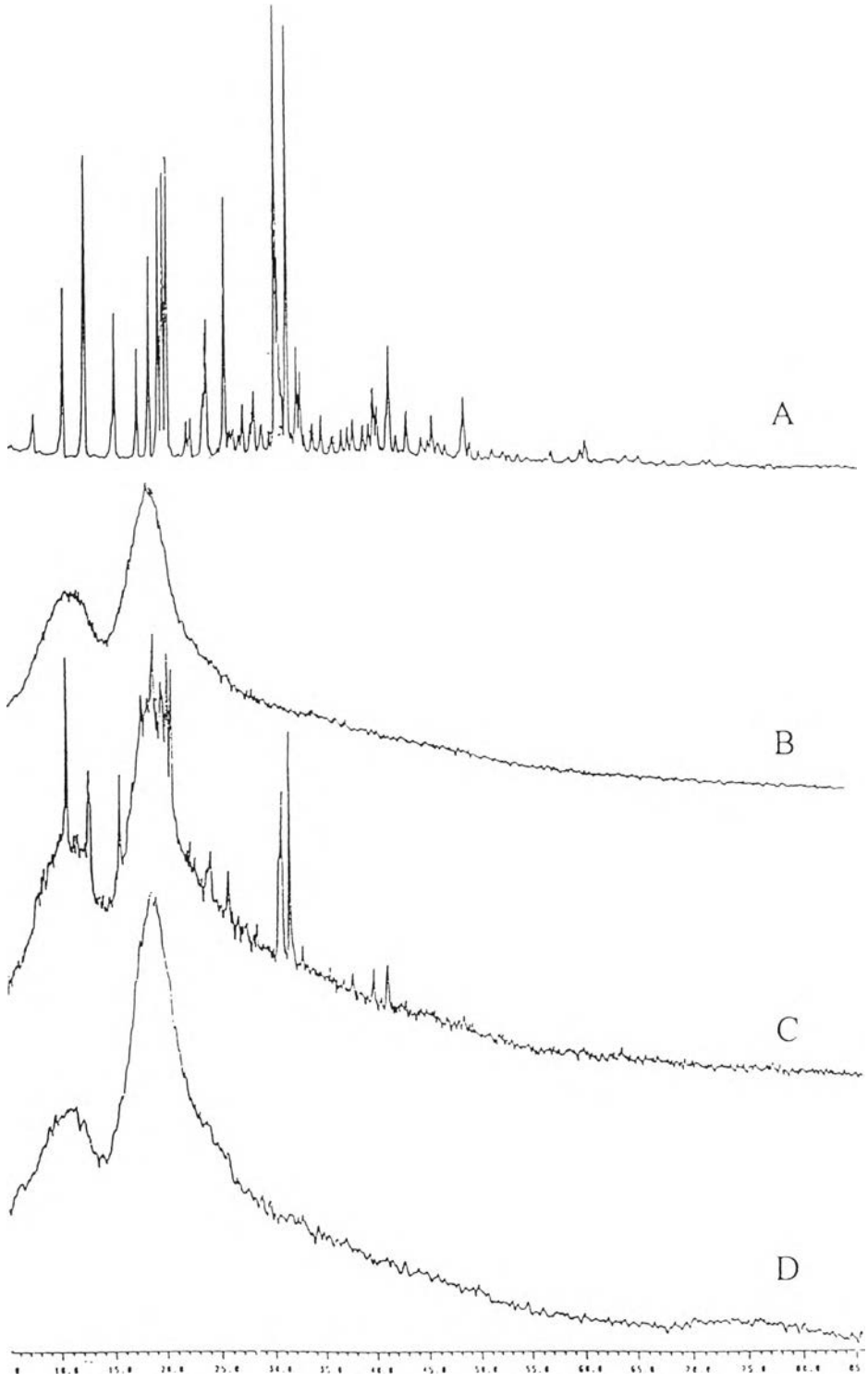

Figure 14 Comparison of X-ray diffraction pattern.
A = Chloramphenicol
B = 2-HP-β-CD
C = Physical mixture (1 : 2)
D = Chloramphenicol : 2-HP-β-CD complex

It could be noticed that the viscosity values were very low (2-5 cps.) and slightly changed after storage.

2. pH measurement

Table 9 showed pH value of various preparations after storage at room temperature and at 45 °C/75% RH in both solution and in powder forms for 4 months. The pH values of all preparations slightly increased.

3. Tonicity measurement

Tonicity of all preparation were listed in Table 10. The tonicity values were between 280-325 milliosmoles. The tonicity of complex solution was the highest whereas the tonicity of BPC 1973 was the lowest. Nevertheless, it could be found that the tonicity value of each preparation did not significantly change when kept for 4 months at various conditions.

Stability Studies of Chloramphenicol : 2-HP-β-CD Complex and Chloramphenicol

1. Chromatographic conditions

Designed chromatographic conditions of chloramphenicol and chloramphenicol : 2-HP-β-CD complex, e.g., mixture of methanol and distilled water in the ratio of 60 : 40 as mobile phase and the wavelength of 254 nm of UV detector, could provide good resolution between chloramphenicol : 2-HP-β-CD complex or chloramphenicol and propyl paraben (internal standard). The chromatogram of complex (Figure 15) was similar to the chromatograms of chloramphenicol (Figure 16). The general characteristics of the chromatograms stated that the base line was smooth, the runtime per sample was within 12 minutes. The retention time of complex or chloramphenicol was about 4 minutes and retention time of propyl paraben was about 8 minutes.

2. Calibration curve

Figure 17 and 18 showed a calibration curve of chloramphenecol : 2-HP-β-CD complex and chloramphenicol, respectively. The calibration curve of chloramphenecol : 2-HP-β-CD complex and chloramphenicol were constructed by plotting the ratio of peak area of complex (or chloramphenicol)

Table 8 Viscosity values of various preparations which kept at room temperature and 45°C/75 % RH for 4 months.

Day	Solution preparations (cps.)				Reconstituted powder which stored at			
					Room temperature		45 °C and 75% RH	
	Formula I *	Formula II **	Complex solution	BPC 1973	Formula I	Formula II	Formula I	Formula II
0	3.903	4.387	3.584	3.918	3.903	4.292	4.143	3.770
1	2.688	2.063	4.131	3.082	-	-	-	-
2	2.795	2.158	4.536	3.151	-	-	-	-
8	2.718	2.267	3.276	3.228	-	-	-	-
20	3.282	2.934	4.150	3.911	-	-	-	-
30	4.291	3.825	3.775	3.193	3.451	3.455	3.538	3.585
60	3.330	3.631	3.166	3.398	2.331	3.661	3.828	3.222
90	3.122	3.548	3.635	3.875	3.782	3.585	3.983	3.272
120	3.431	4.082	3.431	3.769	3.819	3.531	3.690	3.182

* Reconstituted powder (Formula I) for eye drops after reconstitution with water for injection.

** Reconstituted powder (Formula II) for eye drops after reconstitution with its vehicle.

Table 9 pH values of various preparations which kept at room temperature and 45°C/75 % RH for 4 months.

Day	Solution preparations				Reconstituted powder which stored at			
					Room temperature		45 °C and 75% RH	
	Formula I *	Formula II **	Complex solution	BPC 1973	Formula I	Formula II	Formula I	Formula II
0	7.55	7.60	7.50	7.50	7.55	7.50	7.55	7.60
1	7.50	7.50	7.50	7.50	-	-	-	-
2	7.50	7.50	7.50	7.55	-	-	-	-
8	7.58	7.50	7.50	7.50	-	-	-	-
20	7.60	7.60	7.50	7.60	-	-	-	-
30	7.55	7.50	7.50	7.60	7.60	7.55	7.55	7.60
60	7.70	7.70	7.70	7.70	7.55	7.60	7.55	7.65
90	7.70	7.70	7.70	7.70	7.60	7.60	7.60	7.70
120	7.70	7.70	7.70	7.70	7.75	7.70	7.75	7.75

* Reconstituted powder (Formula I) for eye drops after reconstitution with water for injection.

** Reconstituted powder (Formula II) for eye drops after reconstitution with its vehicle.

Table 10 Tonicity values of various preparations which kept at room temperature and 45°C/75 % RH for 4 months.

Day	Solution preparations (Osmole/kg)				Reconstituted powder which stored at			
	Formula I *	Formula II **	Complex solution	BPC 1973	Room temperature		45 °C and 75% RH	
					Formula I	Formula II	Formula I	Formula II
0	0.301	0.304	0.325	0.283	0.301	0.306	0.306	0.305
1	0.302	0.311	0.321	0.280	-	-	-	-
2	0.302	0.306	0.321	0.281	-	-	-	-
8	0.305	0.308	0.324	0.280	-	-	-	-
20	0.304	0.305	0.323	0.282	-	-	-	-
30	0.304	0.304	0.324	0.281	0.306	0.302	0.305	0.308
60	0.306	0.305	0.323	0.284	0.305	0.302	0.304	0.308
90	0.307	0.303	0.324	0.283	0.308	0.308	0.309	0.314
120	0.307	0.306	0.325	0.285	0.309	0.313	0.315	0.312

* Reconstituted powder (Formula I) for eye drops after reconstitution with water for injection.

** Reconstituted powder (Formula II) for eye drops after reconstitution with its vehicle.

Figure 15 Chromatogram of chloramphenicol : 2-HP-β-CD complex.

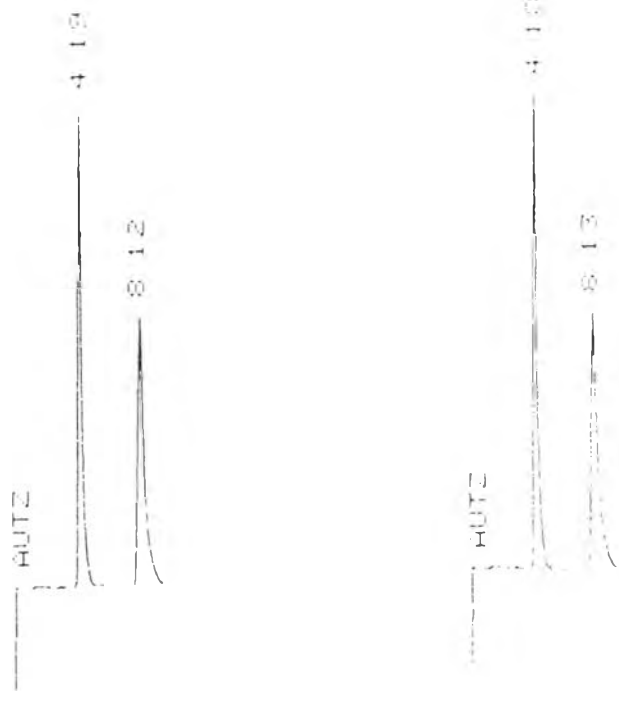

Figure 16 Chromatogram of chloramphenicol.

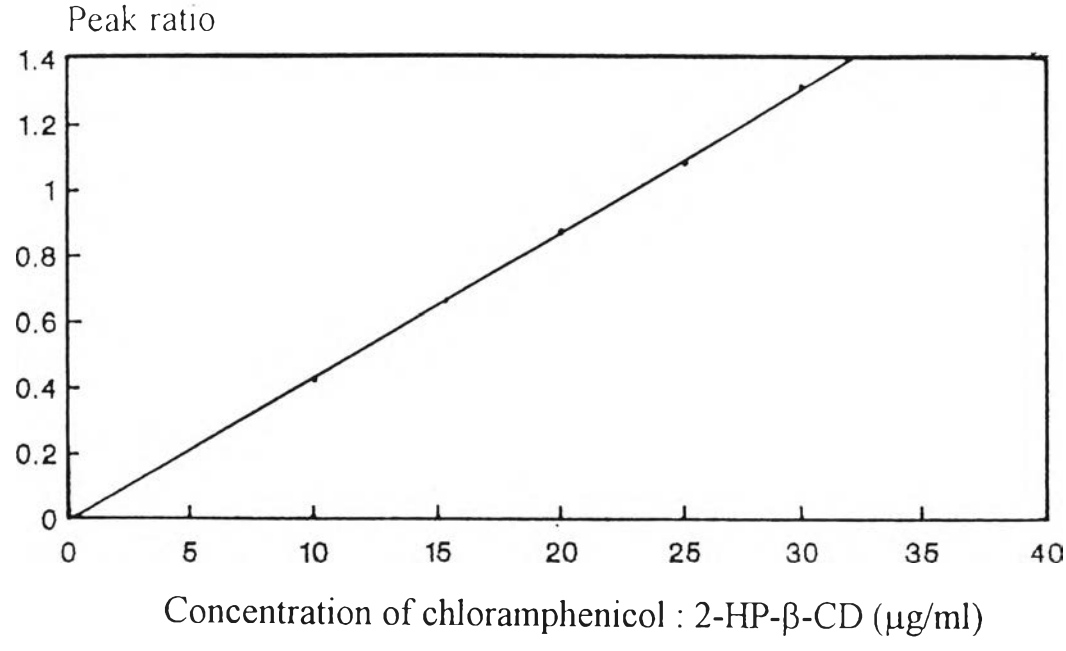

Figure 17 Standard curve of chloramphenicol : 2-HP-β-CD complex.

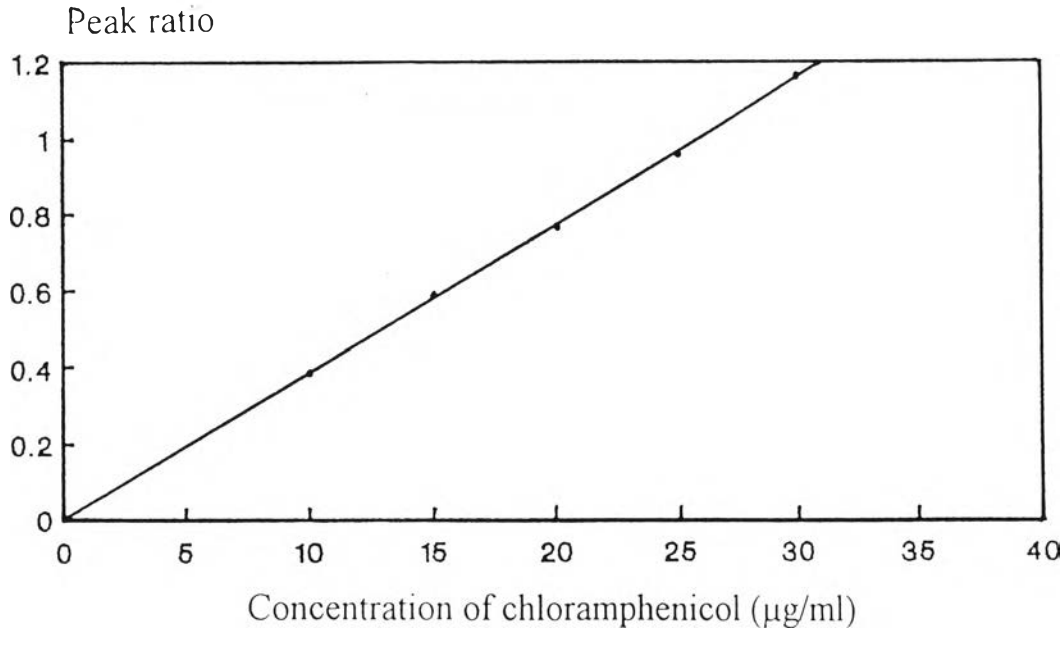

Figure 18 Standard curve of chloramphenicol.

and propyl paraben (internal standard) against the complex (or chloramphenicol) concentration. The calibration curves showed a linear relationship between the ratio of AUC and complex or chloramphenicol concentration and the coefficient of determination were 0.99990 and 0.99991, respectively. The calibration plots were repeated every courses of the analysis.

3. Stability study at 65 °, 55 °, 45 °, 37 °C and room temperature

The accelerated thermodegradation process was performed at 65°, 55°, 45°, 37 °C and at apparent room temperature (25 °C). The degradation data of chloramphenicol in four preparations (Formula I, Formula II, complex solution and BPC 1973) were shown in Table 11-14. Linear regression method was used to evaluate in both of zero order kinetic (concentration versus time) and first order kinetic (ln of concentration versus time). The coefficient of determination (r^2) and other statistic values of four preparations were also listed in Table 15-18. The degradation of all preparations were more fitted to the first order than the zero order. The degradation profiles at various temperatures of four preparations were shown in Figure 19-22. The degradation rate constants (k) were calculated from slope of each linear lines at 65°, 55°, 45°, 37 °C and room temperature.

4. Arrhenius plot, Arrhenius equation and heat of activation

Arrhenius plots of four preparations were plotted between ln k vesus 1/T and shown in Figure 23-26. Arrhenius equation and other statistical values calculated by linear regression analysis were listed in Table 20-23. From the slope of Arrhenius equation, the heat of activation (Ea) was calculated (Appendix I).

Heat of activation can be obtained from slope of Arrhenius plot.The comparison of all heat of activation were listed in Table 24. The heat of activation were between 18 kcal/mole to 25 kcal/mole.The Ea of chloramphenicol eye drop BPC 1973, 18.89 kcal/mole, was the lowest and the Ea of Formula II, 24.89 kcal/mole, was the highest.

5. Rate constant and shelf-life at 25 °C and 8°C

The extrapolated degradation rate constants to 25°C and 8°C of four preparations were calculated from Arrhenius equation. The extrapolated to 25°C and 8°C and apparent degradation rate constants (determine from the degradation profiles of the products kept at room conditions) according to the 90-100 %

Table 11 Five temperatures degradation data of reconstituted powder (Formula I) for eye drops after reconstitution with water for injection.

65 °C			55 °C			45 °C			37 °C			Room Temp. (25 °C)		
Time (day)	%A/A$_0$	ln A/A$_0$	Time (day)	%A/A$_0$	ln A/A$_0$	Time (day)	%A/A$_0$	ln A/A$_0$	Time (day)	%A/A$_0$	ln A/A$_0$	Time (day)	%A/A$_0$	ln A/A$_0$
0	100.58	4.6110	0	101.08	4.6159	0	100.12	4.6064	0	98.98	4.5949	0	100.00	4.6052
1	86.54	4.4606	1	98.10	4.5860	1	99.44	4.5996	1	96.25	4.5670	1	99.94	4.6046
2	84.16	4.4327	2	93.10	4.5337	2	93.18	4.5345	2	95.82	4.5625	2	99.88	4.6040
3	83.66	4.4268	8	78.57	4.3640	8	93.95	4.5428	8	94.80	4.5518	8	99.52	4.6004
4	81.85	4.4049	11	72.53	4.2840	20	83.76	4.4280	20	92.16	4.5235	20	99.94	4.6046
6	73.41	4.2961	13	68.90	4.2326	23	80.48	4.2880	34	90.00	4.4998	34	97.98	4.5848
8	70.80	4.2599	20	55.41	4.0148	27	76.98	4.3436	55	84.21	4.4333	60	96.47	4.5692
11	58.52	4.0694	23	51.92	3.9497	34	71.69	4.2724	60	81.77	4.4039	90	94.75	4.5512
13	53.01	3.9705	27	43.29	3.7680	38	68.78	4.2310	90	78.26	4.3600			
						41	66.39	4.1956						
						44	64.15	4.1612						
						48	63.09	4.1446						

Table 12 Five temperatures degradation data of reconstituted powder (Formula II) for eye drops after reconstitution with its vehicle.*

65 °C			55 °C			45 °C			37 °C			Room Temp. (25 °C)		
Time (day)	%A/A$_0$	ln A/A$_0$	Time (day)	%A/A$_0$	ln A/A$_0$	Time (day)	%A/A$_0$	ln A/A$_0$	Time (day)	%A/A$_0$	ln A/A$_0$	Time (day)	%A/A$_0$	ln A/A$_0$
0	101.02	4.6153	0	100.38	4.6090	0	98.98	4.5949	0	100.15	4.6067	0	100.11	4.6063
1	96.05	4.5649	1	97.61	4.5810	1	94.42	4.5478	1	100.34	4.6086	1	99.94	4.6046
2	90.84	4.5091	2	93.11	4.5338	2	91.02	4.5111	2	100.17	4.6069	2	98.37	4.5887
3	86.69	4.4623	8	82.85	4.4170	8	87.02	4.4662	8	97.75	4.5824	8	99.54	4.6006
4	85.06	4.4434	11	73.20	4.2932	20	78.62	4.3647	20	98.95	4.5946	20	98.87	4.5938
6	79.50	4.3758	13	68.69	4.2296	23	77.28	4.3475	34	94.95	4.5534	34	98.08	4.5858
8	68.39	4.2252	20	54.77	4.0032	27	73.89	4.3026	55	92.75	4.5299	60	96.64	4.5710
11	59.25	4.0818	23	49.07	3.8932	34	71.08	4.2638	60	90.91	4.5099	90	95.00	4.5539
13	55.08	4.0088	27	43.87	3.7812	38	72.63	4.2854	90	85.63	4.4500			
						41	64.62	4.1686						
						44	66.31	4.1944						

* Boric acid, borate and phenylmercuric acetate solution.

Table 13 Five temperatures degradation data of chloramphenicol complex solution.

65 °C			55 °C			45 °C			37 °C			Room Temp. (25 °C)		
Time (day)	$\%A/A_0$	$\ln A/A_0$	Time (day)	$\%A/A_0$	$\ln A/A_0$	Time (day)	$\%A/A_0$	$\ln A/A_0$	Time (day)	$\%A/A_0$	$\ln A/A_0$	Time (day)	$\%A/A_0$	$\ln A/A_0$
0	100.21	4.6073	0	100.00	4.6052	0	103.08	4.6355	0	102.01	4.6251	0	100.13	4.6065
1	96.71	4.5717	1	102.45	4.6294	1	103.50	4.6396	1	102.23	4.6272	1	99.96	4.6048
2	95.34	4.5574	2	98.54	4.5905	2	102.58	4.6306	2	102.02	4.6252	2	99.91	4.6043
3	87.74	4.4744	8	88.68	4.4850	8	98.61	4.5912	8	100.79	4.6130	8	99.64	4.6016
4	85.72	4.4511	11	79.52	4.3760	20	94.75	4.5512	20	98.77	4.5928	20	99.11	4.5963
6	78.26	4.3600	13	71.51	4.2699	23	83.95	4.4302	34	96.66	4.5712	34	98.50	4.5901
8	73.35	4.2952	20	58.85	4.0750	27	80.28	4.3855	55	95.11	4.5550	60	97.38	4.5786
11	65.44	4.1811	23	54.84	4.0045	34	76.98	4.3436	60	90.75	4.5081	90	96.08	4.5652
13	59.25	4.0817	27	45.98	3.8281	38	73.89	4.3026	90	83.11	4.4202			
						41	72.15	4.2787						
						44	70.65	4.2578						
						48	66.77	4.2012						

Table 14 Five temperatures degradation data of chloramphenicol eye drops BPC 1973.

65 °C			55 °C			45 °C			37 °C			Room Temp. (25 °C)		
Time (day)	%A/A$_0$	ln A/A$_0$	Time (day)	%A/A$_0$	ln A/A$_0$	Time (day)	%A/A$_0$	ln A/A$_0$	Time (day)	%A/A$_0$	ln A/A$_0$	Time (day)	%A/A$_0$	ln A/A$_0$
0	99.91	4.6043	0	99.78	4.6030	0	100.06	4.6058	0	101.08	4.6159	0	100.21	4.6073
1	99.24	4.5976	1	98.49	4.5900	1	101.12	4.6163	1	99.76	4.6028	1	100.09	4.6061
2	94.01	4.5434	2	98.23	4.5872	2	98.95	4.5946	2	99.37	4.5989	2	99.92	4.6044
3	89.27	4.4917	8	77.24	4.3469	8	94.25	4.5460	8	98.51	4.5902	8	98.95	4.5946
4	83.70	4.4273	11	67.83	4.2170	20	77.37	4.3486	20	91.14	4.5124	20	97.01	4.5748
6	73.10	4.2918	13	63.73	4.1546	23	74.87	4.3157	34	81.68	4.4028	34	95.98	4.5642
8	60.79	4.1075	20	48.01	3.8715	27	69.78	4.2453	55	71.69	4.2724	60	89.44	4.4936
11	47.28	3.8560	23	43.01	3.7614	34	60.04	4.0950	60	69.53	4.2418	90	86.96	4.4654
13	37.97	3.6369	27	34.84	3.5508	38	62.35	4.1328	90	58.57	4.0703			
						41	57.60	4.0535						
						44	53.95	3.9881						
						48	51.31	3.9379						

Table 15 The statistic values of reconstituted powder (Formula I) for eye drops which were calculated from data presented in Table 11.

Temp. / Statistic value	65 °C		55 °C		45 °C		37 °C		Room Temp. (25 °C)	
	Zero order	First order	Zero order	First order	Zero order	First order	Zero order	First order	Zero order	First order
r^2	0.9760	0.9834	0.9943	0.9983	0.9935	0.9945	0.9870	0.9893	0.9802	0.9805
V.R.*	140.5634	205.6675	613.0400	2033.7114	767.3293	899.4544	264.3060	322.4514	147.2506	149.4242
rate const.(k)**	3.1740	4.3751×10^{-2}	2.0912	3.0067×10^{-2}	0.7756	9.6567×10^{-3}	0.2226	2.5392×10^{-3}	5.9458×10^{-2}	6.1038×10^{-4}
sk***	0.2677	3.0507×10^{-3}	8.4460×10^{-2}	6.6672×10^{-4}	2.8000×10^{-2}	3.2199×10^{-4}	1.3692×10^{-2}	1.4085×10^{-4}	4.8998×10^{-3}	4.9933×10^{-5}
intercept	93.8760	4.5591	98.0532	4.6118	98.6540	4.6008	96.9295	4.5755	100.1579	4.6069

* Variance Ratio

** The rate constant unit for zero order was percent/day and for first order was day^{-1}

*** Standard error of degradation rate constant (slope)

Table 16 The statistic values of reconstituted powder (Formula II) for eye drops which were calculated from data presented in Table 12.

Temp. / Statistic value	65 °C		55 °C		45 °C		37 °C		Room Temp. (25 °C)	
	Zero order	First order	Zero order	First order	Zero order	First order	Zero order	First order	Zero order	First order
r^2	0.9947	0.9961	0.9964	0.9978	0.9757	0.9800	0.9850	0.9839	0.9553	0.9559
V.R.*	659.8725	888.6985	969.4757	1592.8728	178.6487	218.0924	227.4415	211.6820	62.6011	63.6005
rate const.(k)**	3.5370	4.7039×10^{-2}	2.1315	3.0832×10^{-2}	0.6761	8.4184×10^{-3}	0.1570	1.6781×10^{-3}	5.1016×10^{-2}	5.2310×10^{-4}
sk***	0.1377	1.5779×10^{-3}	6.8457×10^{-2}	7.7252×10^{-4}	5.0584×10^{-2}	5.7004×10^{-1}	1.0410×10^{-2}	1.1534×10^{-4}	6.4478×10^{-3}	6.5592×10^{-5}
intercept	99.0732	4.6160	98.5948	4.6198	94.2532	4.5500	100.4441	4.6106	99.6898	4.6021

* Variance Ratio

** The rate constant unit for zero order was percent/day and for first order was day^{-1}

*** Standard error of degradation rate constant (slope)

Table 17 The statistic values of chloramphenicol complex solution which were calculated from data presented in Table 13.

Temp. / Statistic value	65 °C		55 °C		45 °C		37 °C		Room Temp. (25 °C)	
	Zero order	First order	Zero order	First order	Zero order	First order	Zero order	First order	Zero order	First order
r^2	0.9944	0.9976	0.9950	0.9925	0.9874	0.9868	0.9768	0.9713	0.9995	0.9996
V.R.*	615.3733	1428.6928	698.5698	462.9940	388.0748	371.3494	145.6632	116.7822	5667.1980	6789.0991
rate const.(k)**	3.1555	4.0262×10^{-2}	2.1185	2.9181×10^{-2}	0.7849	9.1804×10^{-3}	0.1955	2.0905×10^{-3}	4.4022×10^{-2}	4.4876×10^{-4}
sk***	0.1272	1.0652×10^{-3}	8.0154×10^{-2}	1.3562×10^{-3}	3.9843×10^{-2}	4.7640×10^{-4}	1.6198×10^{-2}	1.9345×10^{-1}	5.8477×10^{-4}	5.4464×10^{-6}
intercept	99.2759	4.6125	102.5352	4.6586	104.3063	4.6561	102.6937	4.6336	100.0218	4.6055

* Variance Ratio

** The rate constant unit for zero order was percent/day and for first order was day^{-1}

*** Standard error of degradation rate constant (slope)

Table 18 The statistic values of chloramphenicol eye drops BPC 1973 which were calculated from data presented in Table 14.

Temp. / Statistic value	65 °C		55 °C		45 °C		37 °C		Room Temp. (25 °C)	
	Zero order	First order	Zero order	First order	Zero order	First order	Zero order	First order	Zero order	First order
r^2	0.9977	0.9904	0.9940	0.9980	0.9944	0.9953	0.9952	0.9983	0.9898	0.9899
V.R.*	1529.9475	358.9360	578.2143	1780.7000	878.7640	1059.6300	720.5399	2075.5777	289.8767	291.4469
rate const.(k)**	5.0384	7.5972×10^{-2}	2.5075	3.8987×10^{-2}	1.0706	1.4260×10^{-2}	0.4930	6.1749×10^{-3}	0.1547	1.6516×10^{-3}
sk***	0.1288	4.0100×10^{-3}	0.1043	9.2390×10^{-4}	3.6115×10^{-2}	4.3807×10^{-4}	1.8366×10^{-2}	1.3554×10^{-4}	9.0862×10^{-3}	9.6744×10^{-5}
intercept	103.0124	4.6892	99.3830	4.6418	100.6531	4.6298	100.4937	4.6194	100.2265	4.6082

* Variance Ratio

** The rate constant unit for zero order was percent/day and for first order was day^{-1}

*** Standard error of degradation rate constant (slope)

Table 19 Comparison rate constant (k) of the 4 preparations at different temperatures and the k value were orderly ranked from minimum to maximum.

Preparation	Degradation rate constant (day^{-1})				
	65°C	55°C	45°C	37°C	25°C
Complex solution	4.0262×10^{-2}	2.9181×10^{-2}	9.1804×10^{-3}	2.0905×10^{-3}	4.4876×10^{-4}
Formula I	4.3751×10^{-2}	3.0067×10^{-2}	9.6567×10^{-3}	2.5292×10^{-3}	6.1038×10^{-4}
Formula II	4.7039×10^{-2}	3.0832×10^{-2}	8.4184×10^{-3}	1.6781×10^{-3}	5.2310×10^{-4}
BPC 1973	7.5972×10^{-2}	3.8987×10^{-2}	1.4260×10^{-2}	6.1749×10^{-3}	1.6516×10^{-3}

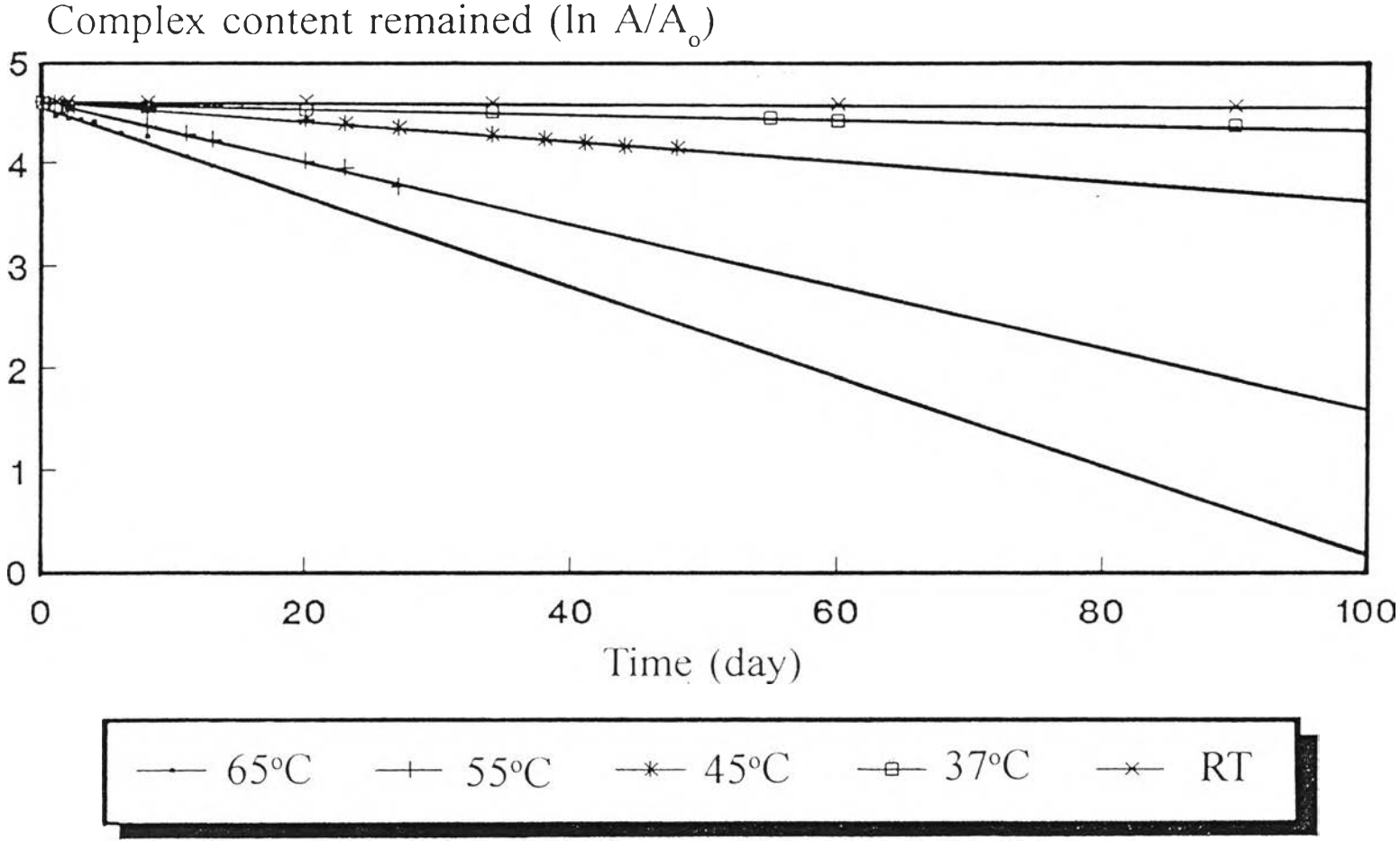

Figure 19 First ordered degradation profiles of reconstituted powder (Formula I) for eye drops.

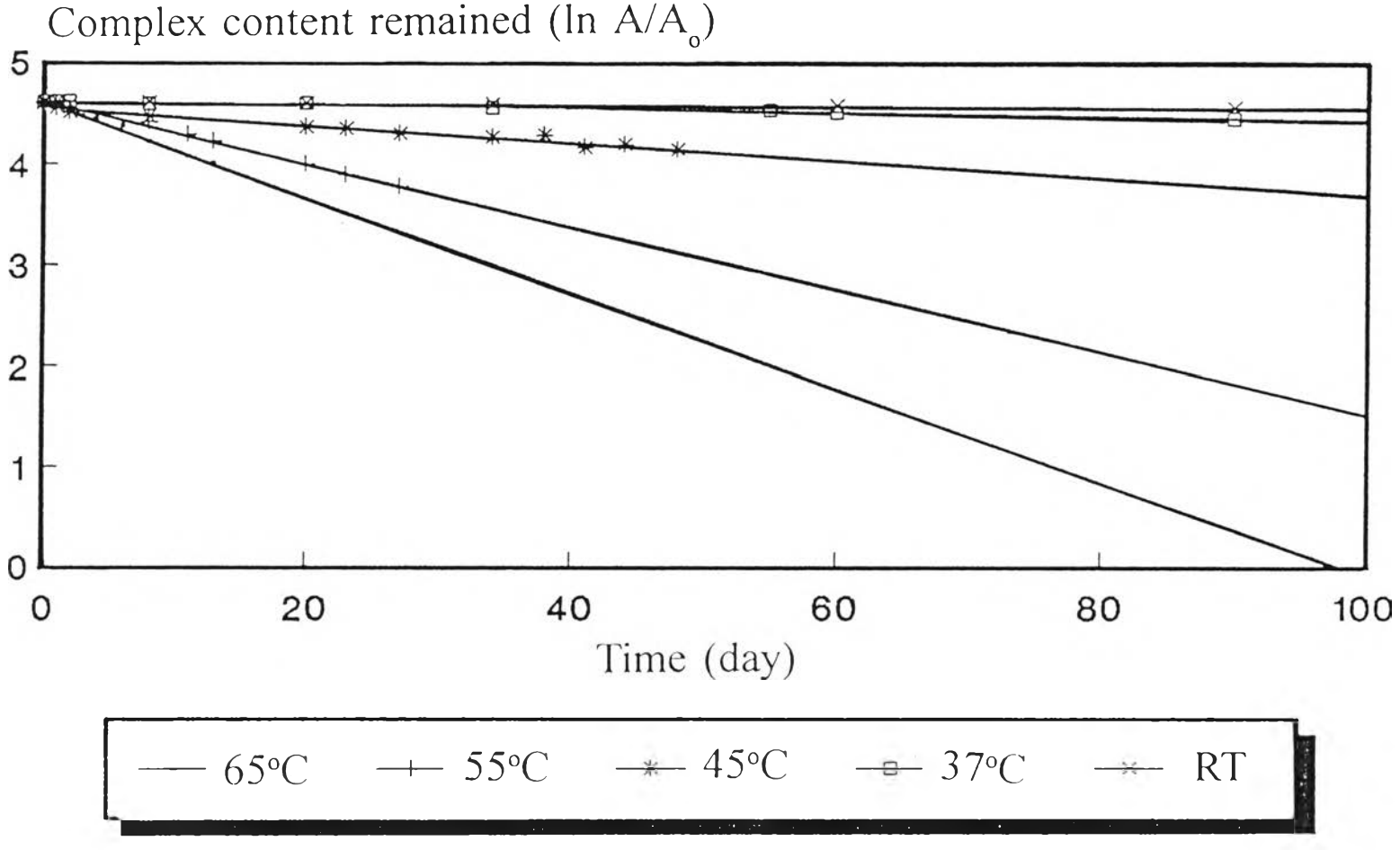

Figure 20 First ordered degradation profiles of reconstituted powder (Formula II) for eye drops.

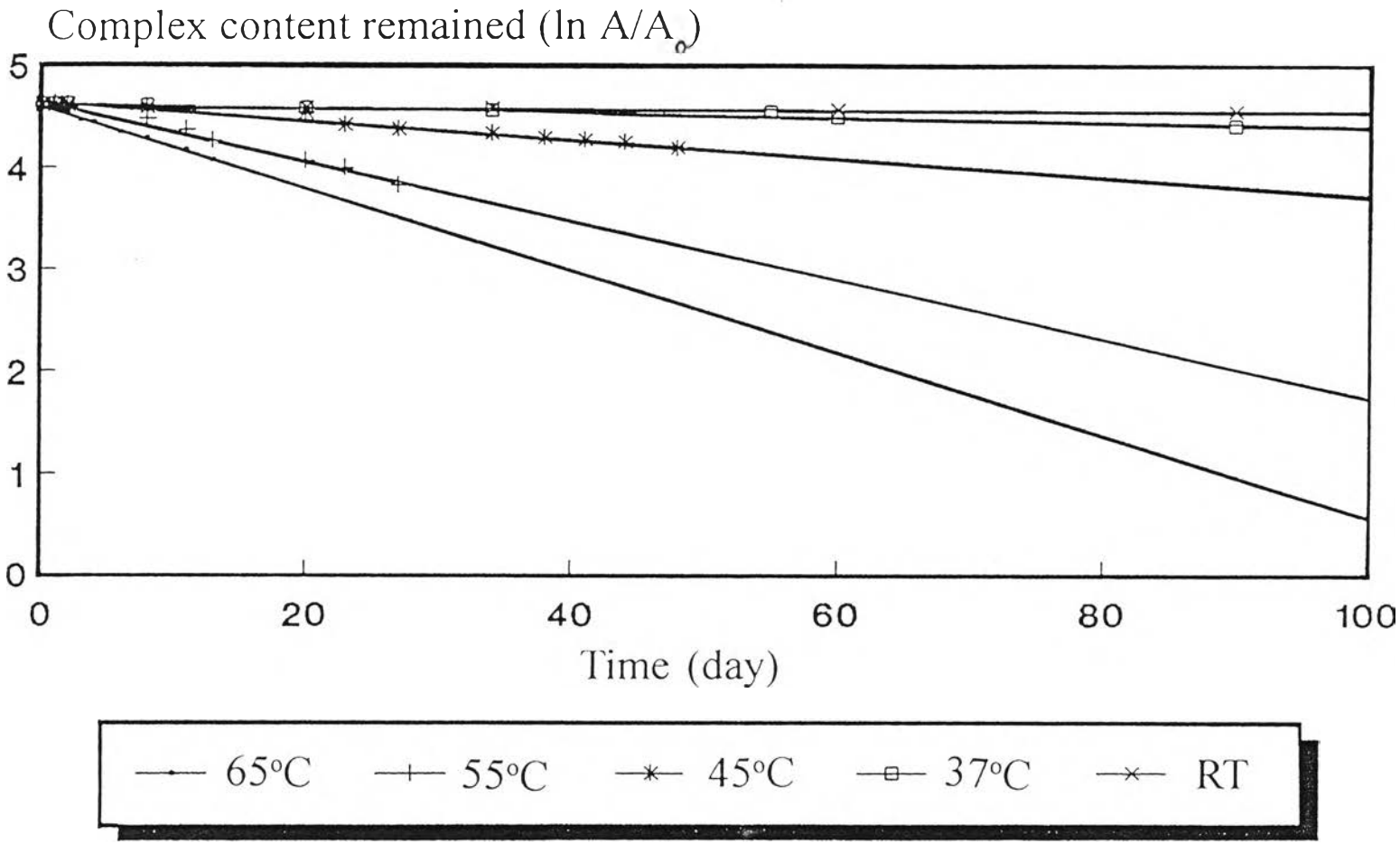

Figure 21 First ordered degradation profiles of chloramphenicol complex solution.

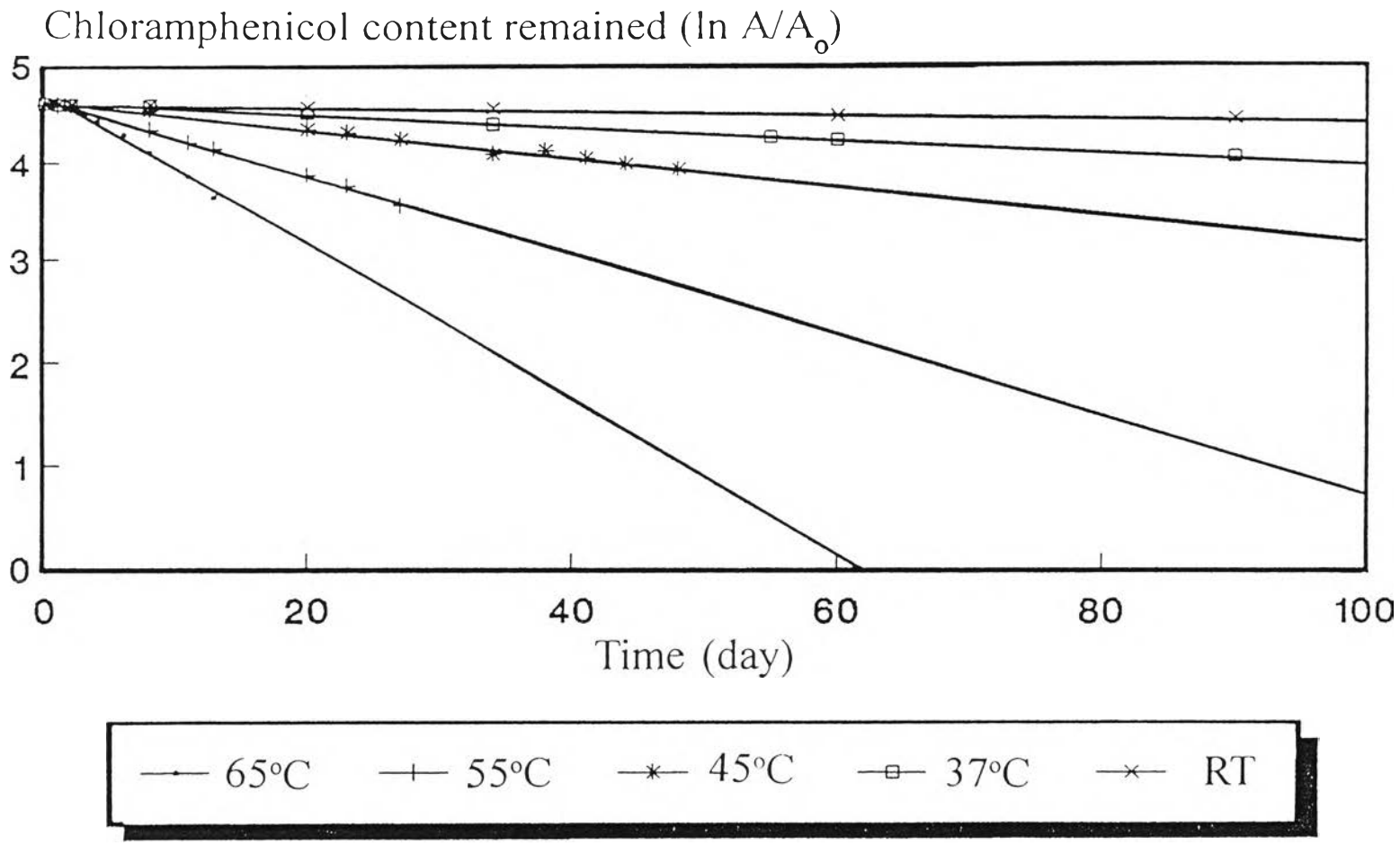

Figure 22 First ordered degradation profiles of chloramphenicol eye drops BPC 1973.

Table 20 Arrhenius relationship of reconstituted powder (Formula I) for eye drops.

Temperature		Degration rate constant of chloramphenical (day^{-1})		
°C	$1/Tx10^3$ K	$k \pm sk \times 10^4$	ln k	ln (k+sk) to ln (k-sk)
65	2.9586	437.51±30.507	-3.1292	-3.0618 to -3.2015
55	3.0488	300.67±6.6672	-3.5043	-3.4824 to -3.5268
45	3.1446	96.567±3.2199	-4.6401	-4.6073 to -4.6740
37	3.2258	25.292±1.4085	-5.9798	-5.9256 to -6.0372
Arrhenius equation	ln k = 28.9347 - 10744.4051(1/T)			
r^2	0.9705			
V.R.	32.3735			
Heat of activation, Ea (cal/mol)	21,349.1329 (21.349 kcal/mol)			
Extrapolated value at 25 °C and 8 °C	25 °C = 3.3557×10^{-3} (K^{-1}) 8 °C = 3.5587×10^{-3} (K^{-1})			
k_{25} $(day)^{-1}$	8.0850×10^{-4}			
k_8 $(day)^{-1}$	9.1295×10^{-5}			
$k_{25} \pm sk$	6.8172×10^{-4} to 9.5886×10^{-4}			
$k_8 \pm sk$	7.6979×10^{-5} to 1.0827×10^{-4}			

Table 21 Arrhenius relationship of reconstituted powder (Formula II) for eye drops.

Temperature		Degration rate constant of chloramphenical (day^{-1})		
°C	$1/Tx10^3$ K	$k \pm sk \times 10^4$	ln k	ln (k+sk) to ln (k-sk)
65	2.9586	470.39±15.779	-3.0568	-3.0238 to -3.0909
55	3.0488	308.32±7.7252	-3.4792	-3.4544 to -3.5046
45	3.1446	84.184±5.7004	-4.7773	-4.7118 to -4.8474
37	3.2258	16.781±1.1534	-6.3901	-6.3236 to -6.4613
Arrhenius equation	ln k = 34.3309 - 12524.6134 (1/T)			
r^2	0.9677			
V.R.	29.4463			
Heat of activation, Ea (cal/mol)	24,886.4068 (24.886 kcal/mol)			
Extrapolated value at 25 °C and 8 °C	25 °C = 3.3557×10^{-3} (K^{-1})			
	8 °C = 3.5587×10^{-3} (K^{-1})			
k_{25} $(day)^{-1}$	4.5376×10^{-4}			
k_8 $(day)^{-1}$	3.5697×10^{-5}			
$k_{25} \pm sk$	3.7964×10^{-4} to 5.4234×10^{-4}			
$k_8 \pm sk$	2.9867×10^{-5} to 4.2666×10^{-5}			

Table 22 Arrhenius relationship of chloramphenicol complex solution.

Temperature		Degration rate constant of chloramphenical (day^{-1})		
°C	$1/Tx10^3$ K	k±sk x 10^4	ln k	ln (k+sk) to ln (k-sk)
65	2.9586	402.62±10.652	-3.2123	-3.1862 to -3.2392
55	3.0488	291.81±13.562	-3.5342	-3.4888 to -3.5818
45	3.1446	91.804±4.764	-4.6907	-4.6401 to -4.7440
37	3.2258	20.905±1.9345	-6.1704	-6.0818 to -6.2674
Arrhenius equation	ln k = 29.9915 - 11114.5352 (1/T)			
r^2	0.9621			
V.R.	24.9080			
Heat of activation, Ea (cal/mol)	22084.5814 (22.084 kcal/mol)			
Extrapolated value at 25 °C and 8 °C	25 °C = 3.3557×10^{-3} (K^{-1}) 8 °C = 3.5587×10^{-3} (K^{-1})			
k_{25} $(day)^{-1}$	6.7180×10^{-4}			
k_8 $(day)^{-1}$	7.0366×10^{-5}			
k_{25}±sk	5.5401×10^{-4} to 8.1464×10^{-4}			
k_8±sk	5.8028×10^{-5} to 8.5327×10^{-5}			

Table 23 Arrhenius relationship of chloramphenicol eye drops BPC 1973.

Temperature		Degration rate constant of chloramphenical (day^{-1})		
°C	$1/Tx10^3$ K	$k \pm sk \times 10^4$	ln k	ln (k+sk) to ln (k-sk)
65	2.9586	759.72±40.100	-2.5774	-2.5260 to -2.6316
55	3.0488	389.87±9.2390	-3.2445	-3.2211 to -3.2685
45	3.1446	142.60±4.3807	-4.2503	-4.2200 to -4.2815
37	3.2258	39.784±0.9571	-5.0873	-5.5031 to -5.5512
Arrhenius equation	ln k = 25.6229 - 9505.0061 (1/T)			
r^2	0.9971			
V.R.	342.0628			
Heat of activation, Ea (cal/mol)	18,886.4471 (18.886 kcal/mol)			
Extrapolated value at 25 °C and 8 °C	25 °C = 3.3557×10^{-3} (K^{-1})			
	8 °C = 3.5587×10^{-3} (K^{-1})			
k_{25} $(day)^{-1}$	1.8864×10^{-3}			
k_8 $(day)^{-1}$	2.7395×10^{-4}			
$k_{25} \pm sk$	1.7874×10^{-3} to 1.9910×10^{-3}			
$k_8 \pm sk$	2.5957×10^{-4} to 2.9812×10^{-4}			

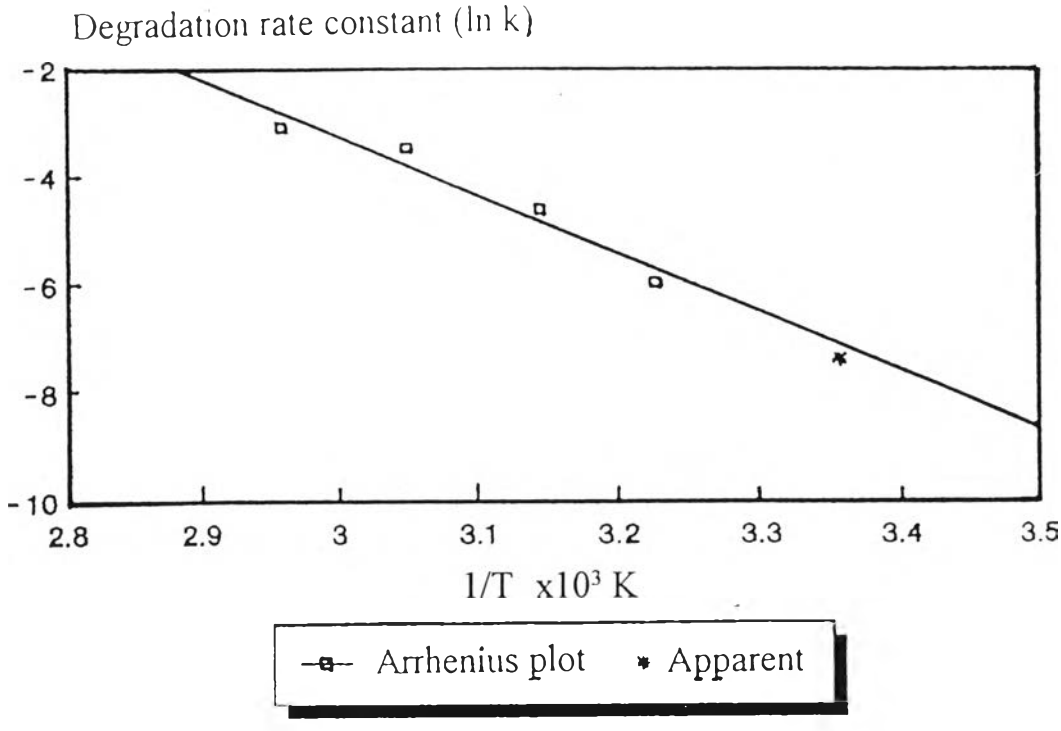

Figure 23 Arrhenius plot of reconstituted powder (Formula I) for eye drops.

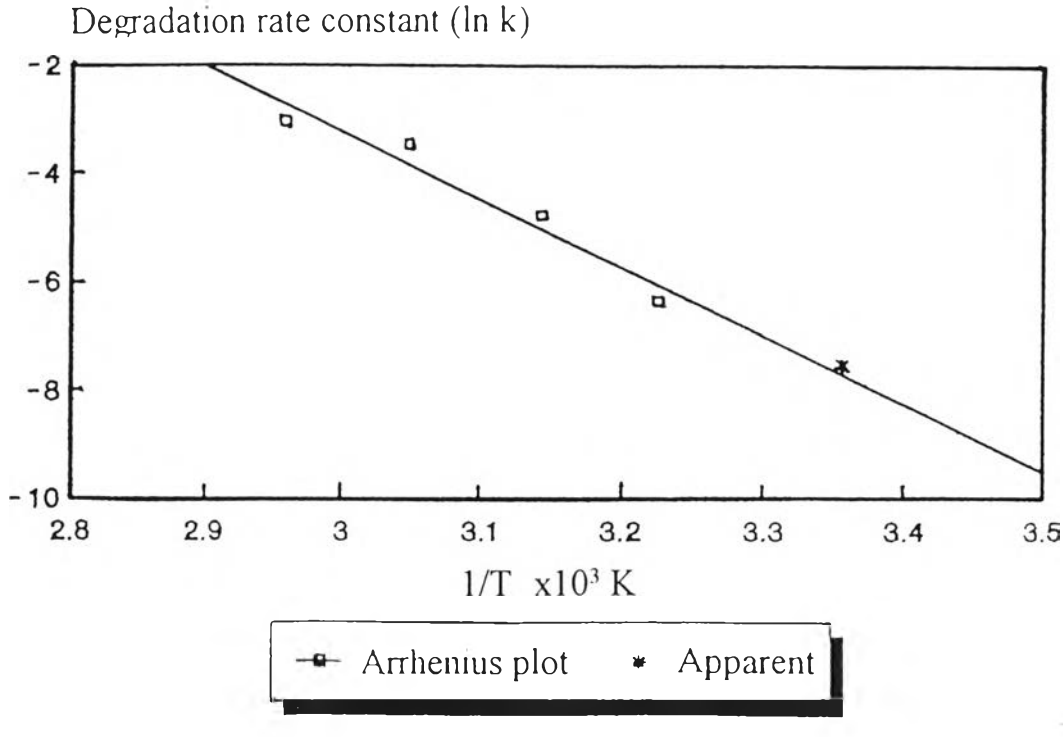

Figure 24 Arrhenius plot of reconstituted powder (Formula II) for eye drops.

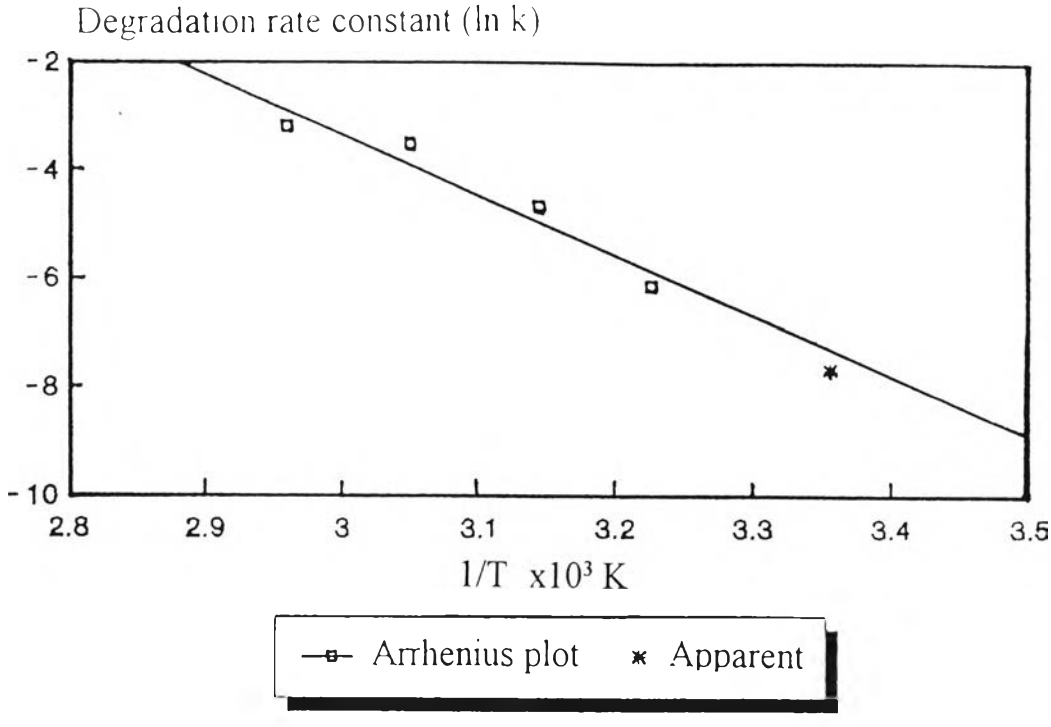

Figure 25 Arrhenius plot of chloramphenicol complex solution.

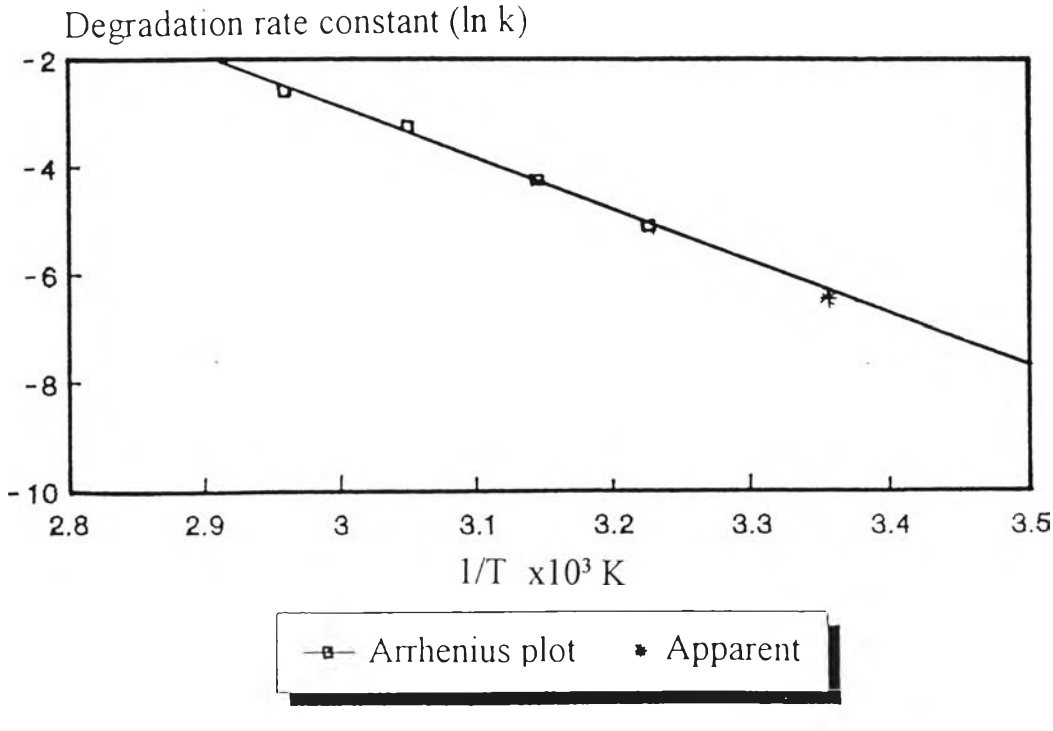

Figure 26 Arrhenius plot of chloramphenicol eye drops BPC 1973.

Table 24 Comparison of heat of activation (Ea).

Formulation	Heat of activation (Ea) (kcal/mole)
Formula I	21.349
Formula II	24.886
Complex solution	22.084
BPC 1973	18.886

labelled amount (LA) and the standard of BP 1993 and corresponding shelf-life of four preparations were compared in Table 25-27, respectively.The orderly rank of the extrapolated to 25°C and 8°C degradation rate constants of each formulation were as follow : BPC 1973 > Formula I, complex solution > Formula II. In the case of the apparent degradation rate constant at room condition, the orderly rank were BPC 1973 > Formula I > Formula II > complex solution.

The shelf-life of four preparations were calculated according to the 90-100 % LA ($t_{90\text{-}100\,\%}$)and the standard of BP 1993 ($t_{90\text{-}110\,\%}$) (Appendix I). At 25 °C and 8°C the rank order of extrapolated and apparent shelf-life were opposite to the order of the degradation rate constant. The extrapolated and apparent shelf-life at 25°C that calculated according to the 90-100 % LA and the standard of BP 1993 were compared in Table 29 and 30. The orderly rank of extrapolated shelf-life ($t_{90\text{-}100\,\%}$) of both conditions were Formula II > complex solution > Formula I > BPC 1973. In the case of apparent shelf-life, the orderly rank were complex solution ~ Formula II > Formula I > BPC1973. The orderly rank of extrapolated and apparent shelf-life that were calculated according to standard of BP 1993 were similar to $t_{90\text{-}100\,\%}$.

6. Solid state stability of reconstituted powder for eye drops of chloramphenicol

Solid state stability of reconstituted powder for eye drops of chloramphenicol (Formula I and Formula II) were studied when storaged at 45°C/75% RH and room temperature. Saturated salt solution of sodium chloride was used to maintained the relative humidity of 75% at the temperature of 45°C. The content of chloramphenicol was determined at various time interval up to 4 months except the products which were kept at room temperature, the condition up to 7 months storage were observed. It was found that the amount of drug in the samples which were kept at both conditions retained the potency above the required lower labelled amount limit. It appeared that the drug content of the products kept at room temperature remained unchanged. Figure 27-28 showed comparison of Formula I and II at both conditions.

Table 25 Comparison of the degradation rate constants extrapolated to 25°C and shelf-life calculated according to the 90 - 100 % LA and the standard of BP 1993.

Formulation	Degradation rate constant extrapolated to 25 °C		Shelf-life at 25 °C according to 90-100% LA		Shelf-life at 25 °C according to BP 1993 (90-110% LA)	
	$k_{25} \times 10^4$ (day^{-1})	interval of $k_{25} \times 10^4$ (day^{-1})	$t_{90\text{-}100}$ (month)	interval of $t_{90\text{-}100}$	$t_{90\text{-}110}$ (month)	interval of $t_{90\text{-}110}$
Formula I	8.0850	6.8172 to 9.5886	4.3455	3.6641 to 5.1536	8.27	6.98 to 9.81
Formula II	4.5376	3.7964 to 5.4234	7.7427	6.4781 to 9.2544	14.74	12.34 to 17.62
Complex solution	6.7180	5.5401 to 8.1464	5.2297	4.3127 to 6.3416	9.96	8.21 to 12.08
BPC 1973	18.8640	17.874 to 19.910	1.8624	1.7646 to 1.9656	3.55	3.36 to 3.74

Table 26 Comparison of the apparent degradation rate constants at room temperature and shelf-life calculated according to the 90 - 100 % LA and the standard of BP 1993.

Formulation	Degradation rate constant at 25 °C		Shelf-life at 25 °C according to 90-100% LA		Shelf-life at 25 °C according to BP 1993 (90-110% LA)	
	$k_{25} \times 10^4$ (day^{-1})	interval of $k_{25} \times 10^4$ (day^{-1})	$t_{90\text{-}100}$ (month)	interval of $t_{90\text{-}100}$	$t_{90\text{-}110}$ (month)	interval of $t_{90\text{-}110}$
Formula I	6.1038	5.6045 to 6.6031	5.7560	5.3207 to 6.2688	10.96	10.13 to 11.94
Formula II	5.2310	4.5751 to 5.8869	6.7164	5.7680 to 7.6792	12.79	11.36 to 14.62
Complex solution	4.4876	4.4331 to 4.5421	7.8290	7.7350 to 7.9252	14.91	14.73 to 15.09
BPC 1973	16.5160	15.5486 to 17.4834	2.1272	2.0095 to 2.2596	4.05	3.83 to 4.30

Table 27 Comparison of the degradation rate constants extrapolated to 8°C and shelf-life calculated according to the 90 - 100 % LA and the standard of BP 1993.

Formulation	Degradation rate constant extrapolated to 8 °C		Shelf-life at 8 °C according to 90-100% LA		Shelf-life at 8 °C according to BP 1993 (90-110% LA)	
	$k_8 \times 10^5$ (day^{-1})	interval of $k_8 \times 10^5$ (day^{-1})	t_{90-100} (month)	interval of t_{90-100}	t_{90-110} (month)	interval of t_{90-110}
Formula I	9.1295	7.6979 to 10.827	38.4833	32.4497 to 45.6402	73.2789	61.7900 to 86.9068
Formula II	3.5697	2.9867 to 4.2666	98.421	82.3450 to 117.6326	187.4107	156.7993 to 223.9930
Complex solution	7.0366	5.8028 to 8.5327	49.9294	41.1749 to 60.5455	95.0743	78.4042 to 115.2892
BPC 1973	27.3950	25.9570 to 28.9120	12.8247	12.1518 to 13.5352	24.4205	23.1392 to 25.7734

Table 28 Comparison of the extrapolated degradation rate constants and apparent rate constants at room temperature.

Preparation	Extrapolated degradation rate constant (day^{-1})	Apparent degradation rate constant (day^{-1})
Formula I	8.0850×10^{-4}	6.1038×10^{-4}
Formula II	4.5376×10^{-4}	5.2310×10^{-4}
complex solution	6.7180×10^{-4}	4.4876×10^{-4}
BPC 1973	1.8864×10^{-3}	1.6516×10^{-3}

Table 29 Comparison the extrapolated shelf-life and the apparent shelf-life according to the 90 - 100 % LA at room temperature.

Formulation	Extrapolated shelf-life (months)	Apparent shelf-life (months)
Formula I	3.6641 to 5.1536	5.3207 to 6.2688
Formula II	6.4781 to 9.2544	5.9680 to 7.6792
Complex solution	4.3127 to 6.3416	7.7350 to 7.9252
BPC 1973	1.7646 to 1.9656	2.0095 to 2.2596

Table 30 Comparison the extrapolated shelf-life and the apparent shelf-life according to the standard of BP 1993 at room temperature.

Formulation	Extrapolated shelf-life (months)	Apparent shelf-life (months)
Formula I	6.98 to 9.81	10.13 to 11.94
Formula II	12.34 to 17.62	11.36 to 14.62
Complex solution	8.21 to 12.08	14.73 to 15.09
BPC 1973	3.36 to 3.74	3.83 to 4.30

Table 31 The solid state stability of reconstituted powder for eye drops when kept at room temperature and 45°C and 75 % RH.

Day	Formula I (%)		Formula II (%)	
	45 °C 75% RH	Room Temp	45 °C 75% RH	Room Temp.
0	99.74	100.86	100.04	100.21
31	100.31	99.40	100.64	99.61
60	99.34	99.92	100.59	98.81
90	95.65	99.34	99.18	98.40
120	96.95	99.57	97.81	99.38
210	-	102.74	-	99.13

Antimicrobial Activity Test of Chloramphenicol and Complex

Agar diffusion assay was used for antimicrobial activity test of free chloramphenicol base and chloramphenicol : 2-HP-β-CD complex. Each of concentrations was compared with the 50 μg/ml of chloramphenicol reference standard. Table 32 showed the inhibition zone diameter of reference standard, chloramphenicol and chloramphenicol : 2-HP–β–CD complex. Figure 29 showed the standard curve of chloramphenicol. The sample concentrations were obtained by reading from the standard curve. The concentration of free chloramphenicol and complex were found to be 51.0 μg/ml and 52.0 μg/ml, respectively.

Eye Irritability Test of Chloramphenicol and Chloramphenicol : 2-HP-β-CD Eye Drops in Rabbit

This experiment was designed to study the irritability of chloramphenicol and chloramphenicol : 2-HP-β-CD eye drops using albino rabbits as test animals and compared to the effects of 0.9% sodium chloride solution for injection. The results of eye irritation were showed in Table 33.

In all control group (n=2), 0.9% sodium chloride solution for injection did not cause eye irritation and inflammation. In the case of all tested rabbits (n=8), chloramphenicol and chloramphenicol : 2-HP-β-CD eye drops did not cause corneal cloudiness or opacity. Both solutions did not also cause edema, congestion or hemorrhage of iris, did not alter pupillary light reflex.

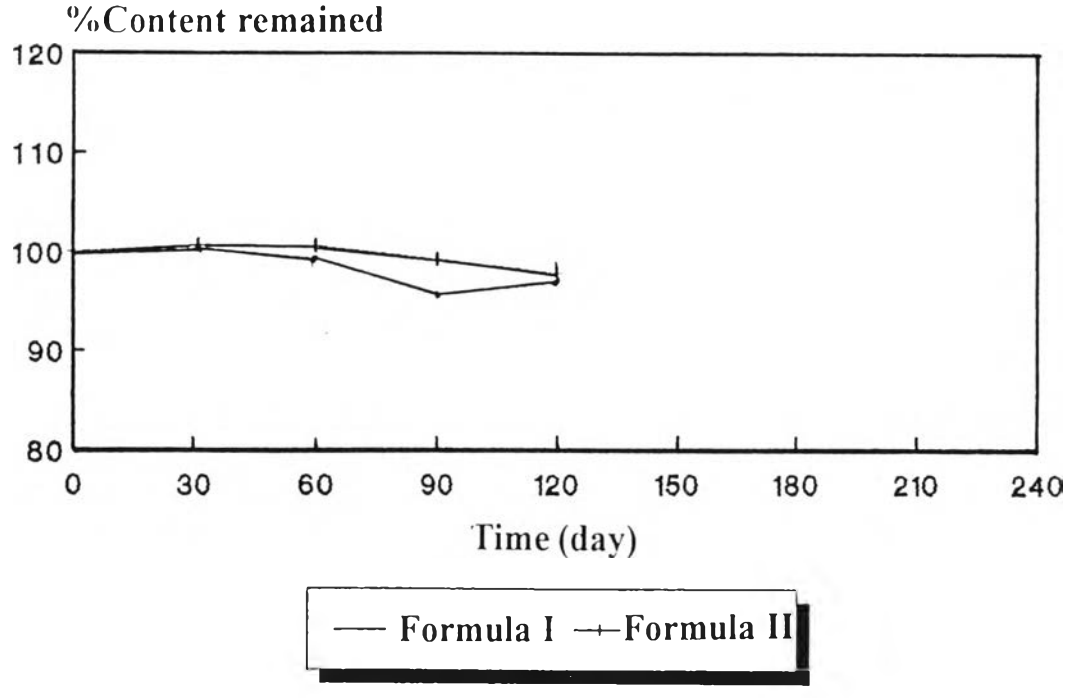

Figure 27 Comparison of stability of reconstituted powder for eye drops at 45°C and 75 % RH for 4 months.

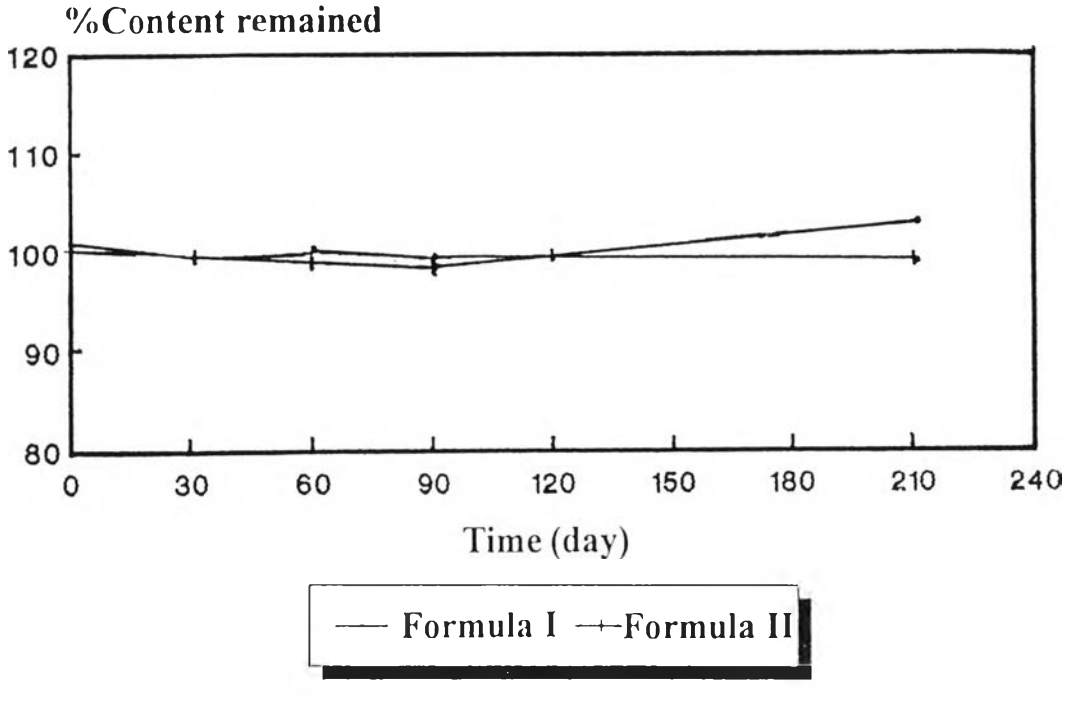

Figure 28 Comparison of stability of reconstituted powder for eye drops at room temperature for 7 months.

Table 32 The inhibition zone diameter (mm) of chloramphenicol and chloramphenicol : 2-HP-β-CD.

	Standard								Chloramphenicol		Chloramphenicol : HP-β-CD complex	
Concentration	12.0	50.0	40.0	50.0	62.5	50.0	78.1	50.0	Chloramphenicol 50.0 ug/ml	Standard 50.0 ug/ml	Complex 50.0 ug/ml	Standard 50.0 ug/ml
A	17.20	22.00	19.18	22.16	24.44	22.58	25.94	22.78	21.96	22.34	22.88	22.86
	17.00	22.10	19.68	22.60	24.46	22.22	25.90	22.48	21.98	22.36	23.22	22.86
	17.22	22.00	20.42	22.20	23.90	22.42	26.58	22.46	22.60	22.16	22.96	23.50
B	17.66	22.18	20.20	22.14	24.02	22.58	25.34	22.58	21.70	22.60	22.98	22.40
	17.10	22.90	19.56	22.24	24.52	22.08	25.58	22.20	23.44	22.34	22.92	22.40
	17.44	22.12	20.04	22.22	24.46	22.32	26.58	22.18	23.02	22.40	22.92	22.46
C	17.30	22.90	19.72	22.36	25.40	23.06	25.20	22.24	23.00	21.74	22.40	22.34
	17.42	22.78	19.76	23.42	24.00	22.46	26.00	22.88	22.32	22.76	22.52	22.76
	17.42	22.52	20.00	23.64	24.54	22.34	26.68	22.58	22.40	22.80	22.76	22.14
Total	155.76	201.50	178.56	202.98	219.74	202.06	233.80	202.38	202.42	201.50	205.56	203.72
Mean	17.31	22.39	19.84	22.55	24.42	22.45	25.98	22.49	22.49	22.39	22.84	22.64
Correction zone ϕ of 50 ug/ml	0.08		-0.08		0.02		-0.02		0.08		-0.17	
Corrected value	17.39		19.76		24.44		25.96		22.57		22.67	

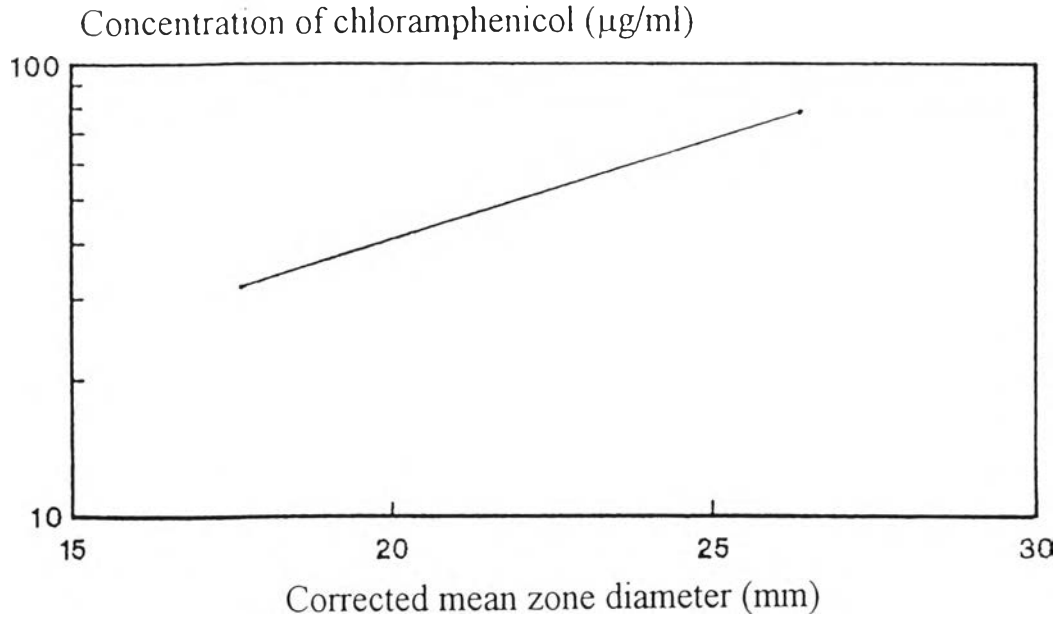

Figure 29 The standard curve of chloramphenicol for antimicrobial activity test of chloramphenicol and chloramphenicol : 2-HP-β-CD complex.

Table 33 Eye irritation test of chloramphenicol and chloramphenicol : 2-HP-β-CD complex

Reaction	Control group	Tested group	
	n=2	Chloramphenicol (n=4)	Complex (n=4)
Cornea:			
• cloundiness, ulceration	0	0	0
Iris:			
• congestion, red hemorrhage, edema	0	0	0
Conjunctiva:			
• edema, red of the eyelids and nictitating membrane	0	0	0