



## CHAPTER III

### RESULTS

#### 3.1 Tonicity of formulations

The tonicity of each formulation was listed in Table 4 and illustrated in Figure 3. Additives incorporated into the standard formulation (BPC 1973, pH = 7.0) increased the tonicity of the formulation. The degree of increment was orderly ranked by the following PEG > PF<sub>407</sub> > HPMC > PVP. Both HPMC and PVP slightly increased the tonicity. Molecular weight of the additives also affected the tonicity. PEG of low molecular weight increased the tonicity more than those of high molecular weight. From the tonicity measurement of 37 formulations in Table 4, candidate formulations were chosen according to

1. Limitation of tonicity. It is stated that tonicity of eye drops should not be more than 630 milliosmoles (Appendix B).
2. Higher amount of cosolvent. Because of partial replacement of water by a cosolvent was expected to decrease hydrolysis, formulations with more amount of cosolvent were chosen.

Sixteen formulations were further studied.

#### 3.2 Analysis of Chloramphenicol

Chromatograms of the standard solution of CPC and

Table 4 The tonicity of prepared CPC formulations.

No.	Formulation	Tonicity (milliosmoles)	Different tonicity value from BPC
1	BPC*	263.5	0
2	BPC ,pH =6*	259.5	-4
3	0.1% HPMC*	276.0	12.5
4	0.4% HPMC*	280.5	17.0
5	0.8% HPMC*	282.5	19.0
6	1% PVP*	274.5	11.0
7	4% PVP*	284.5	21.0
8	8% PVP*	296.0	32.5
9	10% PEG <sub>400</sub>	747.0	483.5
10	15% PEG <sub>400</sub>	1099.0	835.5
11	20% PEG <sub>400</sub>	1608.0	1344.5
12	25% PEG <sub>400</sub>	Unmeasurable	-
13	10% PEG <sub>1500</sub>	435.0	171.5
14	15% PEG <sub>1500</sub>	651.0	351.5
15	20% PEG <sub>1500</sub>	875.0	611.5
16	25% PEG <sub>1500</sub>	Unmeasurable	-
17	10% PEG <sub>4000</sub>	378	114.5
18	15% PEG <sub>4000</sub>	516	252.5
19	20% PEG <sub>4000</sub>	734	470.5
20	25% PEG <sub>4000</sub>	Unmeasurable	-
21	10% PEG <sub>6000</sub>	367	103.5
22	15% PEG <sub>6000</sub>	491	227.5
23	20% PEG <sub>6000</sub> *	704	440.5
24	20% PEG <sub>6000</sub> pH=6.0*	704	440.5
25	25% PEG <sub>6000</sub> *	1067	803.5
26	25% PEG <sub>6000</sub> pH=6.0*	1067	803.5
27	30% PEG <sub>6000</sub>	1,725	1,488.5
28	40% PEG <sub>6000</sub>	Unmeasurable	-
29	10% PEG <sub>20000</sub>	358.5	95.0
30	15% PEG <sub>20000</sub>	461.5	198.0
31	20% PEG <sub>20000</sub> *	707.5	444.0
32	25% PEG <sub>20000</sub> *	1076.0	812.5
33	30% PEG <sub>20000</sub> *	Unmeasurable	-
34	40% PEG <sub>20000</sub>	Unmeasurable	-
35	5% PF <sub>407</sub>	296.5	33.0
36	10% PF <sub>407</sub>	360.0	96.5
37	15% PF <sub>407</sub> *	474.0	210.5

\* selected formulations

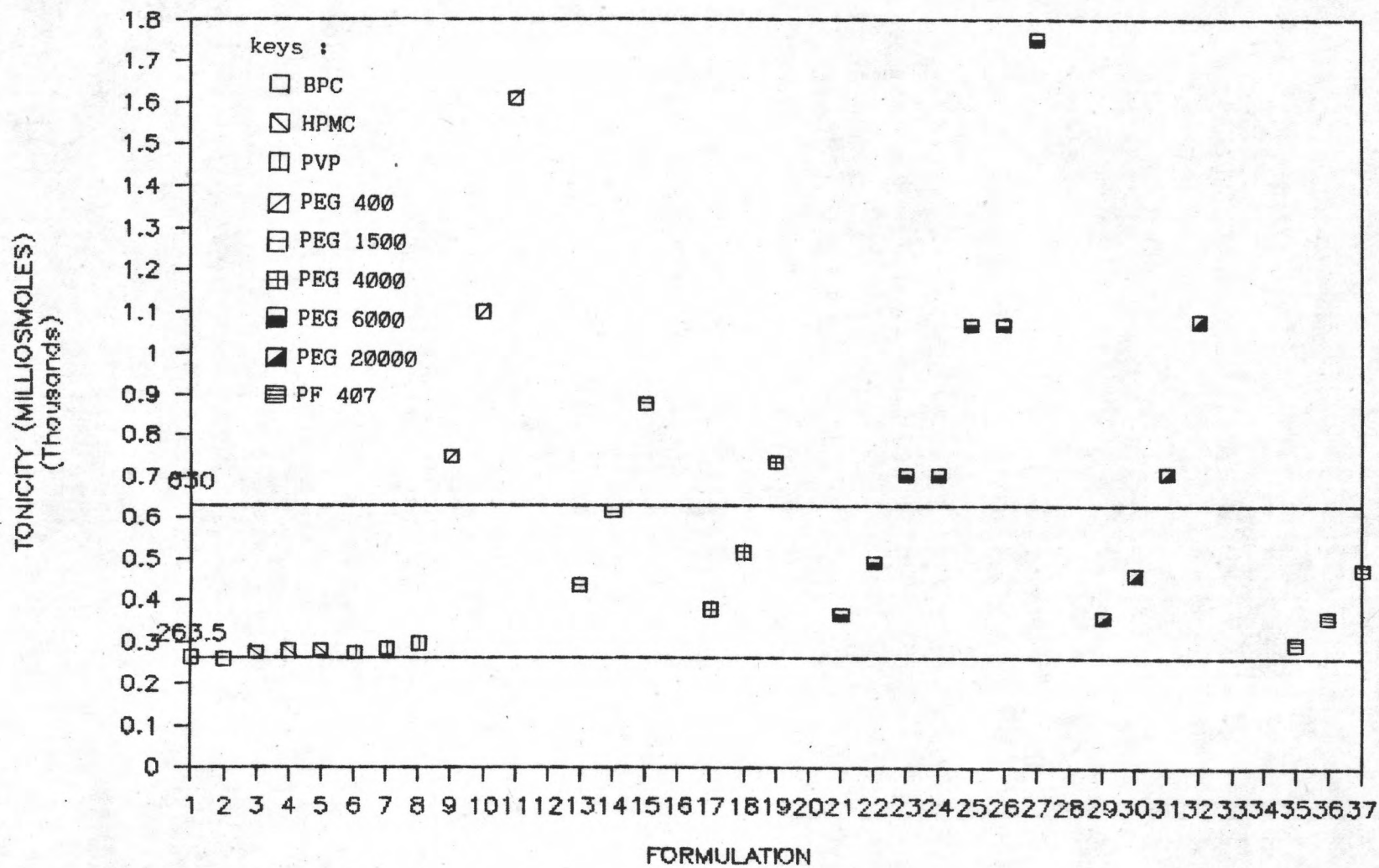


Figure 3 Comparison of the tonicity of all CPC formulations, 263.5 milliosmoles was the tonicity of standard formulation and 630 milliosmoles was the upper tolerance limit.



propylparaben (PP) as the internal standard were shown in Figure 4 . The first pair of chromatograms was at the beginning of analysis , the second pair was at the middle of analysis and the third pair was at the end of analysis. It was seen that the peak area ratios of CPC/PP in different times were slightly different. The general characteristics of the chromatograms stated that the base line was smooth , the retention time of CPC was about 4 minutes and retention time of PP was about 9 minutes.

Chromatograms of the six formulations were shown in Figures 5-10. The retention times of CPC and PP were also approximately 4 minutes and 9 minutes. The chromatograms of CPC indicated that the peak area ratios of CPC/PP decreased which meant that the concentrations were decreased after incubation as shown in Figures 5-10.

### 3.3 Stability Data at 60°C.

#### 3.3.1 Order of Reaction Rate.

The degradations of CPC in sixteen feasible formulations (BPC, BPC pH = 6.0, 1% PVP, 4% PVP, 8% PVP, 0.1% HPMC, 0.4% HPMC, 0.8% HPMC, 20% PEG<sub>6000</sub>, 20% PEG<sub>6000</sub> pH = 6.0, 25% PEG<sub>6000</sub>, 25% PEG<sub>6000</sub> pH = 6.0, 20% PEG<sub>20000</sub>, 25% PEG<sub>20000</sub>, 30% PEG<sub>20000</sub>, 15% PF<sub>407</sub>) were shown in Tables 5-20. The data include of the relationship between concentration and time. Linear regression method was used to evaluate in both of zero order (concentration versus time) and first order (ln of concentration versus time) kinetics. The coefficient of determination ( $r^2$ ) and other statistic values were also listed. It



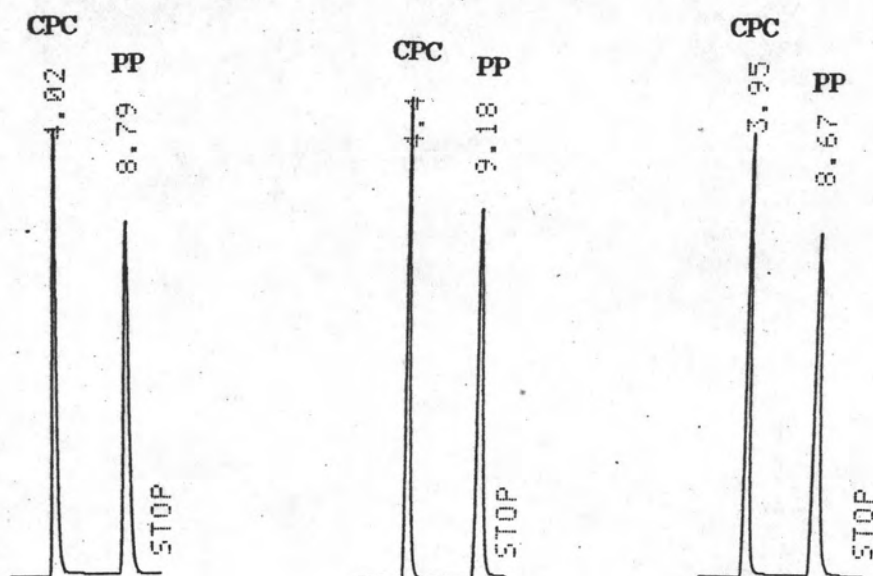


Figure 4 Chromatograms of the standard solution of CPC and PP as the internal standard.

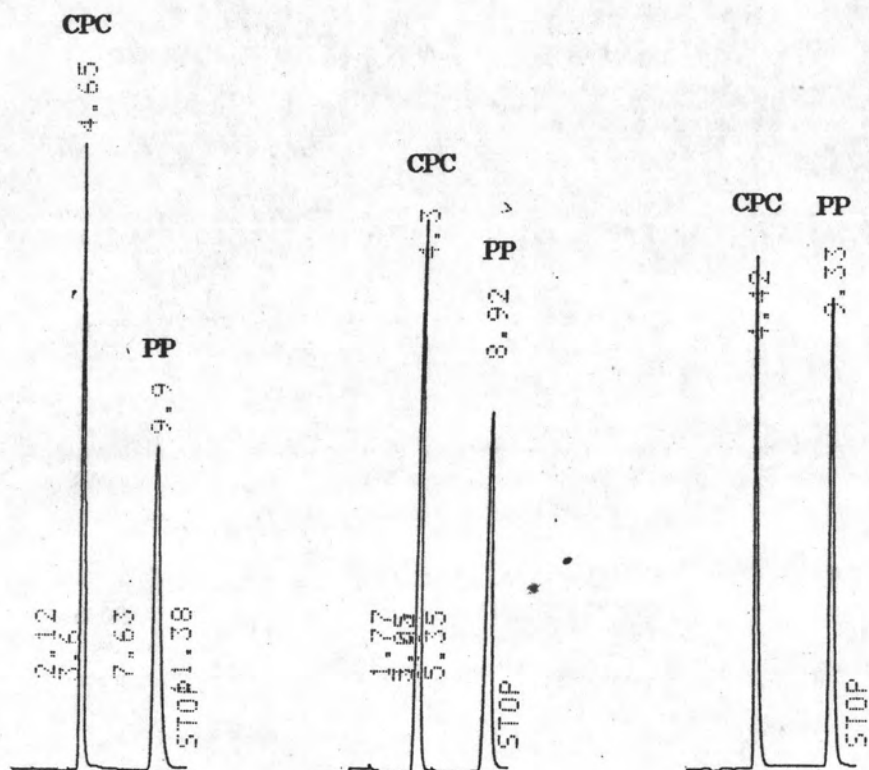


Figure 5 Chromatograms of CPC and PP in CPC eye drops BPC 1973.

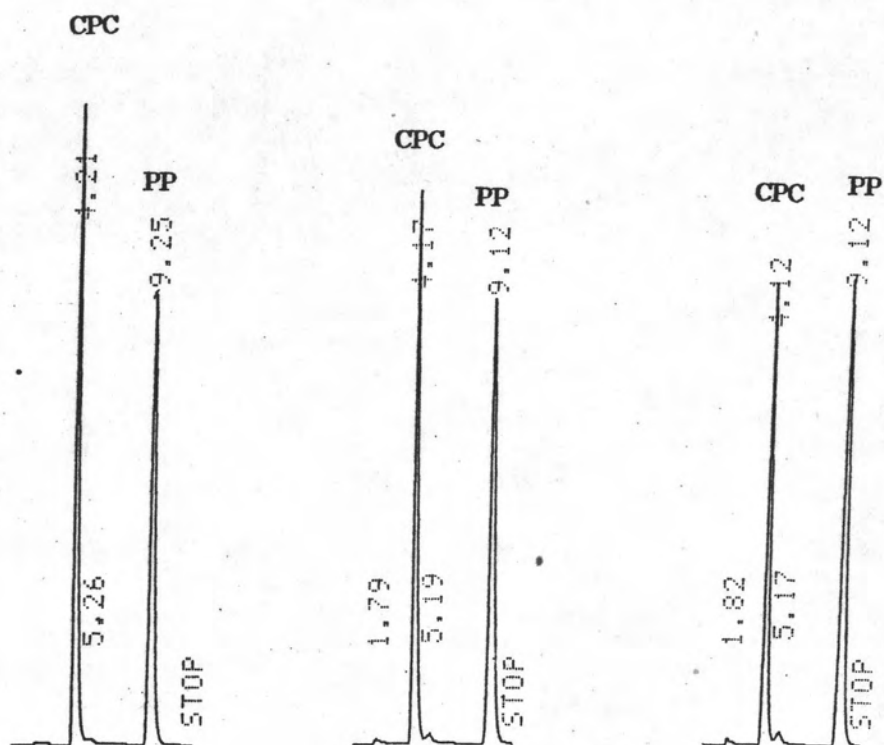


Figure 6 Chromatograms of CPC and PP in CPC eye drops BPC 1973 containing 0.4% HPMC.

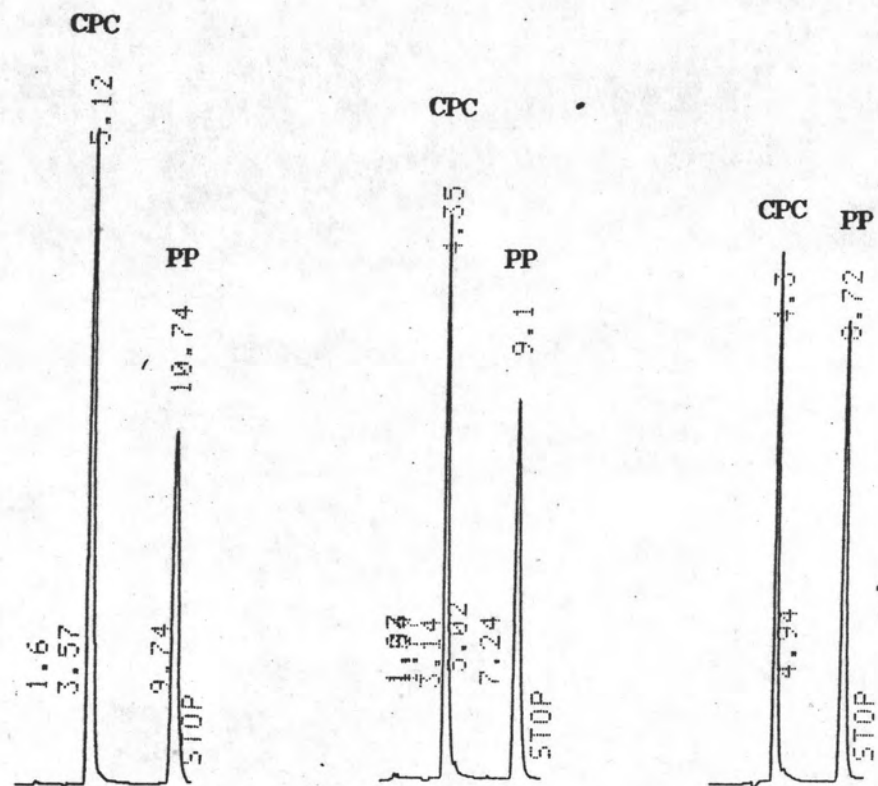


Figure 7 Chromatograms of CPC and PP in CPC eye drops BPC 1973 containing 1% PVP.



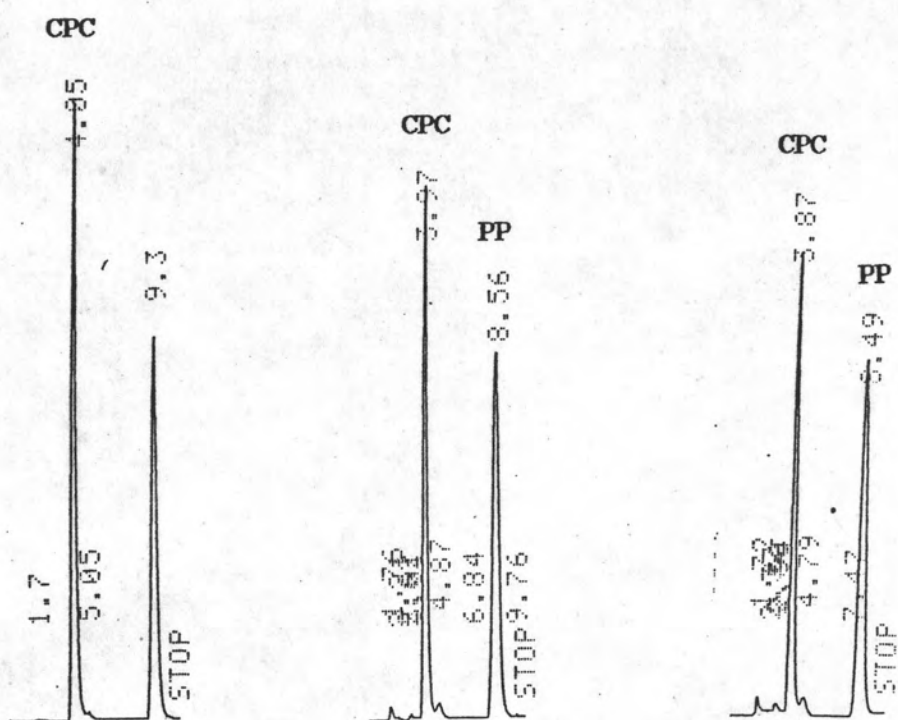


Figure 8 Chromatograms of CPC and PP in CPC eye drops BPC  
1973 containing 25% PEG<sub>6000</sub>.

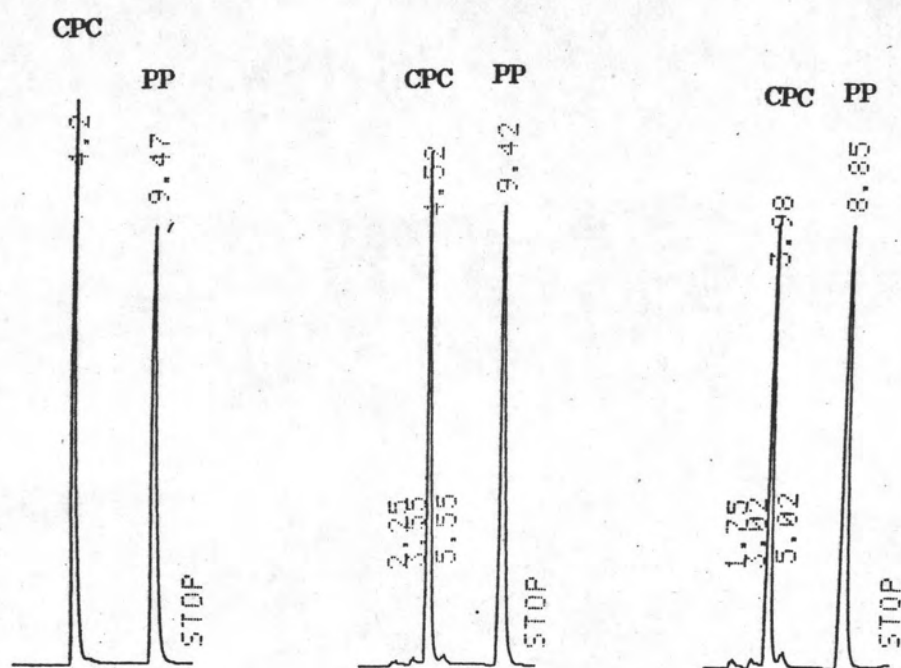


Figure 9 Chromatograms of CPC and PP in CPC eye drops BPC 1973 containing 25% PEG<sub>20000</sub>.

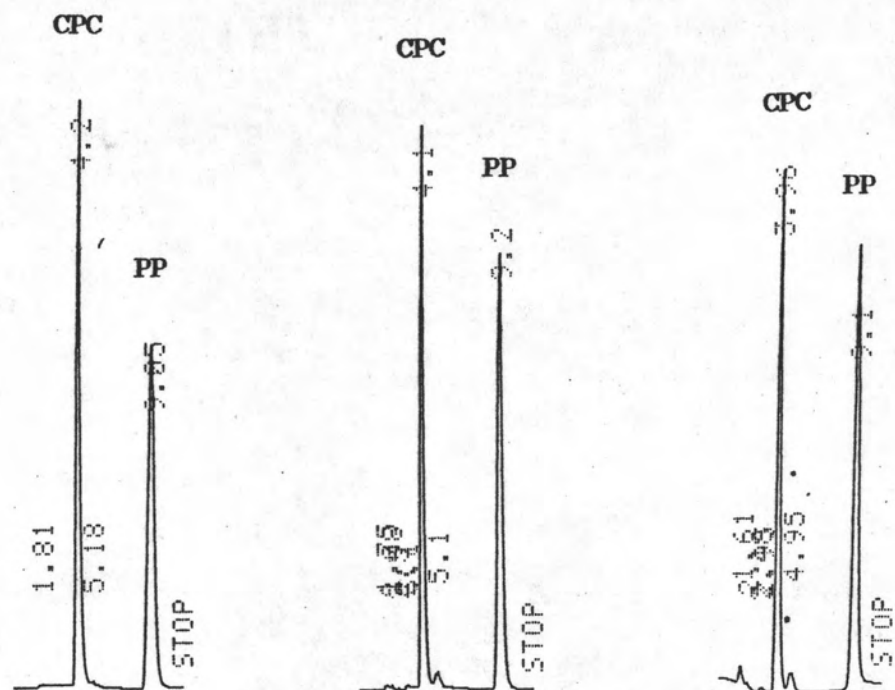


Figure 10 Chromatograms of CPC and PP in CPC eye drops BPC 1973 containing 15% PF<sub>407</sub>.

Table 5 Degradation data of CPC eye drops BPC at 60°C.

Formulation	Time (day)	CPC Concentration (mg/100 ml)	ln Concentration
BPC, pH = 7.0 60°C	0	454.52	6.1192
	7	338.34	5.8241
	10	288.04	5.6631
	13	259.08	5.5571
	21	183.63	5.2129
	24	153.76	5.0354
	27	148.94	5.0035
Statistic Value	Zero Order	First Order	
r <sup>2</sup>	0.9636	0.9950	
V.R.	132.1967	999.8187	
d.f.	1,5	1,5	
reject Ho with P	0.0004	0.0001	
rate constant (k)*	-11.1254	-4.2770 E-02	
std.error of k(Sb)	±0.9676	±1.3526 E-02	
intercept.	423.0137	6.1111	

\* The rate constant unit for zero order was mg% / day and for first order was (day<sup>-1</sup>).



Table 6 Degradation data of CPC eye drops BPC adjusted to pH = 6.0 at 60°C.

Formulation	Time (day)	CPC Concentration (mg/100 ml)	ln Concentration
BPC, pH = 6.0 60°C	0	430.19	6.0642
	3	393.37	5.9748
	7	324.00	5.7807
	10	285.06	5.6527
	14	233.68	5.4540
	18	201.98	5.3082
	21	175.16	5.1657
	25	143.09	4.9635
	28	127.33	4.8468
Statistic Value	Zero Order	First Order	
$r^2$	0.9756	0.9988	
V.R.	280.4082	5597.4240	
d.f.	1,7	1,7	
Reject $H_0$ with P	0	0	
rate constant (k)*	-10.9520	-4.4307 E-02	
std.error of k (Sb)	$\pm 0.6543$	$\pm 5.9222$ E-04	
intercept	410.4234	6.0881	

\* The rate constant unit for zero order was mg% / day and for first order was ( $\text{day}^{-1}$ ).



Table 7 Degradation data of CPC eye drops BPC containing 1% PVP at 60°C.

Formulation	Time (day)	CPC Concentration (mg/100 ml)	ln Concentration
1% PVP , 60°C	0	470.68	6.1542
	4	384.27	5.9513
	7	348.02	5.8523
	10	296.01	5.6904
	13	257.99	5.5529
	17	242.36	5.4904
	21	193.13	5.2634
	24	163.85	5.0990
	27	155.13	5.0443
Statistic Value	Zero Order	First Order	
$r^2$	0.9605	0.9919	
V.R.	170.0742	856.1098	
d.f.	1,7	1,7	
reject Ho with P	0.0001	0	
rate constant (k)*	-11.2486	-4.1239 E-02	
std.error of k(Sb)	+0.8625	+1.4094 E-03	
intercept	432.7803	6.1301	

\* The rate constant unit for zero order was mg% / day and for first order was (day<sup>-1</sup>).

Table 8 Degradation data of CPC eye drops BPC containing 4%PVP at 60°C.

Formulation	Time (day)	CPC Concentration (mg/100 ml)	ln Concentration
4% PVP , 60°C	0	330.61	5.8009
	4	283.21	5.6462
	7	247.62	5.5119
	10	207.63	5.3357
	17	145.48	4.9800
	20.4167	132.75	4.8885
Statistic Value	Zero Order	First Order	
$r^2$	0.9813	0.9945	
V.R.	210.3256	726.6680	
d.f.	1,4	1,4	
reject Ho with P	0.0007	0.0003	
rate constant (k)*	-9.9027	-4.6849 E-02	
std.error of k(Sb)	+0.6828	+1.7379 E-03	
intercept	320.9641	5.8166	

\* The rate constant unit for zero order was mg% / day and for first order was (day<sup>-1</sup>).

Table 9 Degradation data of CPC eye drops BPC containing 8% PVP at 60°C.

Formulation	Time (day)	CPC Concentration (mg/100 ml)	ln Concentration
8% PVP , 60°C	0	439.2	6.0849
	3	379.39	5.9386
	7	315.84	5.7552
	10	268.38	5.5924
	14	221.53	5.4005
	17	191.25	5.2536
	24	142.71	4.9608
	28	99.02	4.5953
Statistic Value	Zero Order	First Order	
r <sup>2</sup>	0.9680	0.9921	
V.R.	181.5069	750.6535	
d.f.	1,6	1,6	
reject Ho with P	0.0001	0	
rate constant (k)*	-11.6785	-5.0877 E-02	
std.error of k(Sb)	±0.8668	±1.8569 E-03	
intercept	407.5255	6.1027	

\* The rate constant unit for zero order was mg% / day and for first order was (day<sup>-1</sup>).



Table 10 Degradation data of CPC eye drops BPC containing 0.1% HPMC at 60°C.

Formulation	Time (day)	CPC Concentration (mg/100 ml)	ln Concentration
0.1% HPMC , 60°C	0	442.3	6.0920
	3	405.57	6.0053
	7	345.12	5.8439
	11	288.24	5.6638
	14	245.52	5.5034
	17	231.44	5.4443
	22	185.17	5.2213
	25	157.81	5.0614
	29	140.87	4.9478
Statistic Value	Zero Order	First Order	
$r^2$	0.9725	0.9967	
V.R.	247.3513	2111.5840	
d.f.	1,7	1,7	
reject Ho with P	0	0	
rate constant (k)*	-10.6509	-4.0718 E-02	
std.error of k(Sb)	+0.6772	+8.8611 E-04	
intercept	422.8170	6.1106	

\* The rate constant unit for zero order was mg% / day and for first order was (day<sup>-1</sup>).

Table 11 Degradation data of CPC eye drops BPC containing 0.4% HPMC at 60°C.

Formulation	Time (day)	CPC Concentration (mg/100 ml)	ln Concentration
0.4% HPMC , 60°C	0	435.88	6.0774
	3	383.56	5.9495
	7	324.51	5.7823
	10	277.33	5.6252
	14	216.22	5.3763
	18	197.17	5.2841
	21	170.04	5.1360
	24	147.79	4.9958
	28	129.20	4.8614
Statistic Value	Zero Order	First Order	
r <sup>2</sup>	0.9595	0.9950	
V.R.	165.9993	1383.9020	
d.f.	1,7	1,7	
reject Ho with P	0.0001	0	
rate constant (k)*	-11.0154	-4.4346 E-02	
std.error of k(Sb)	+0.8549	+1.1921 E-03	
intercept	406.5133	6.0701	

\* The rate constant unit for zero order was mg% / day and for first order was (day<sup>-1</sup>).

Table 12 Degradation data of CPC eye drops BPC containing 0.8% HPMC at 60°C.

Formulation	Time (day)	CPC Concentration (mg/100 ml)	ln Concentration	mg/100 ml
0.8% HPMC , 60°C	0	471.65	6.1562	
	4	396.10	5.9817	
	8	336.86	5.8197	
	11	288.34	5.6641	
	14	252.51	5.5315	
	17	232.81	5.4502	
	22	186.80	5.2300	
	25	157.93	5.0622	
	29	145.30	4.9788	
Statistic Value	Zero Order		First Order	
r <sup>2</sup>	0.9588		0.9960	
V.R.	163.0724		1748.8820	
d.f.	1,7		1,7	
reject Ho with P	0.0001		0	
rate constant (k)*	-11.1283		-4.1576 E-02	
std.error of k(Sb)	+0.8714		+9.9418 E-04	
intercept	434.9980		6.1421	

\* The rate constant unit for zero order was mg% / day and for first order was (day<sup>-1</sup>).

Table 13 Degradation data of CPC eye drops BPC containing 20% PEG<sub>6000</sub> at 60°C.

Formulation	Time (day)	CPC Concentration (mg/100 ml)	ln Concentration
20% PEG <sub>6000</sub> pH = 7.0 , 60°C	0	444.43	6.0968
	4	384.19	5.9511
	8	335.70	5.8162
	11	299.42	5.7018
	15	254.76	5.5403
	18	240.11	5.4811
	22	206.63	5.3309
	25	177.18	5.1772
	29	166.29	5.1137
Statistic Value	Zero Order	First Order	
r <sup>2</sup>	0.9723	0.9956	
V.R.	245.8943	1570.3090	
d.f.	1,7	1,7	
reject Ho with P	0	0	
rate constant (k)*	-9.6038	-3.4784 E-02	
std.error of k(Sb)	±0.6124	±8.7778 E-04	
intercept	419.6009	6.0890	

\* The rate constant unit for zero order was mg% / day and for first order was (day<sup>-1</sup>).



Table 14 Degradation data of CPC eye drops BPC containing 20% PEG<sub>6000</sub> and adjusted to pH=6.0 at 60°C.

Formulation	Time (day)	CPC Concentration (mg/100 ml)	ln Concentration
20% PEG <sub>6000</sub> pH = 6.0 , 60°C	0	451.34	6.1122
	3	397.77	5.9859
	6	356.94	5.8776
	9	317.01	5.7589
	14	254.66	5.5399
	17	230.44	5.4400
	21	192.65	5.2609
	27	153.07	5.0309
Statistic Value	Zero Order	First Order	
r <sup>2</sup>	0.9774	0.9996	
V.R.	259.7761	13859.7600	
d.f.	1,6	1,6	
reject Ho with P	0.0001	0	
rate constant (k)*	-11.0626	-4.0167 E-02	
std.error of k(Sb)	+0.6864	+3.4119 E-04	
intercept	428.3692	6.1128	

\* The rate constant unit for zero order was mg% / day and for first order was (day<sup>-1</sup>).

Table 15 Degradation data of CPC eye drops BPC containing 25% PEG<sub>6000</sub> and adjusted to pH=6.0, at 60°C.

Formulation	Time (day)	CPC Concentration (mg/100 ml)	ln Concentration
25% PEG <sub>6000</sub> pH = 6.0, 60°C	0	461.06	6.1335
	3	409.84	6.0158
	7	364.24	5.8978
	10	319.55	5.7669
	13	287.58	5.6615
	17	258.28	5.5540
	20	229.45	5.4357
	24	193.06	5.2630
	27	181.82	5.2030
Statistic Value	Zero Order	First Order	
r <sup>2</sup>	0.9792	0.9976	
V.R.	329.2029	2884.6910	
d.f.	1,7	1,7	
reject Ho with P	0	0	
rate constant (k)*	-10.2705	-3.4882 E-02	
std.error of k(Sb)	+0.5661	+6.4947 E-04	
intercept	438.6238	6.1280	

\* The rate constant unit for zero order was mg% / day and for first order was (day<sup>-1</sup>).

Table 16 Degradation data of CPC eye drops BPC containing 25% PEG<sub>6000</sub> at 60°C.

Formulation	Time (day)	CPC Concentration (mg/100 ml)	ln Concentration
25% PEG <sub>6000</sub> pH = 7.0, 60°C	0	441.85	6.0910
	4	389.07	5.9638
	8	335.84	5.8166
	11	309.55	5.7351
	15	269.03	5.5948
	18	238.54	5.4745
	22	218.42	5.3864
	25	190.01	5.2471
	29	165.62	5.1097
Statistic Value	Zero Order	First Order	
r <sup>2</sup>	0.9819	0.9982	
V.R.	378.8621	3798.8000	
d.f.	1,7	1,7	
reject Ho with P	0	0	
rate constant (k)*	-9.3987	-3.3615 E-02	
std error of k(Sb)	+0.4829	+5.4540 E-04	
intercept.	422.0623	6.0951	

\* The rate constant unit for zero order was mg% / day and for first order was (day<sup>-1</sup>).



Table 17 Degradation data of CPC eye drops BPC containing 20% PEG<sub>20000</sub> at 60°C.

Formulation	Time (day)	CPC Concentration	ln Concentration
		(mg/100 ml)	
20% PEG <sub>20000</sub> , 60°C	0	485.48	6.1851
	3	427.69	6.0584
	7	364.07	5.8973
	10	338.08	5.8233
	13.4167	290.30	5.6709
	17	252.85	5.5328
	20	234.93	5.4593
	24	198.23	5.2894
	28	173.52	5.1563
Statistic Value	Zero Order	First Order	
r <sup>2</sup>	0.9724	0.9983	
V.R.	246.7491	4007.6900	
d.f.	1,7	1,7	
reject Ho with P	0	0	
rate constant (k)*	-10.9304	-3.6494 E-02	
std.error of k(Sb)	±0.6958	±5.7646 E-04	
intercept	455.9128	6.1711	

\* The rate constant unit for zero order was mg% / day and for first order was (day<sup>-1</sup>).



Table 18 Degradation data of CPC eye drops BPC containing 25% PEG<sub>20000</sub> at 60°C.

Formulation	Time (day)	CPC Concentration (mg/100 ml)	ln Concentration
25% PEG <sub>20000</sub> , 60°C	0	447.45	6.1036
	6.0208	360.31	5.8870
	10	319.15	5.7657
	14	279.54	5.6331
	20	241.09	5.4852
	24	213.53	5.3638
	27	191.22	5.2534
	29	189.28	5.2432
Statistic Value	Zero Order	First Order	
r <sup>2</sup>	0.9682	0.9956	
V.R.	182.6472	1350.5180	
d.f.	1,6	1,6	
reject Ho with P	0.0001	0	
rate constant (k)*	-8.5722	-2.9756 E-02	
std.error of k(Sb)	±0.6343	±8.0970 E-04	
intercept	419.5165	6.0755	

\* The rate constant unit for zero order was mg% / day and for first order was (day<sup>-1</sup>).

Table 19 Degradation data of CPC eye drops BPC containing 30% PEG<sub>20000</sub> at 60°C.

Formulation	Time (day)	CPC Concentration	ln Concentration
30% PEG <sub>20000</sub> , 60°C	0	470.32	6.1543
	4	426.83	6.0564
	7	382.02	5.9455
	10.4167	357.52	5.8792
	14	316.37	5.7569
	17	284.81	5.6518
	21	264.26	5.5769
	25	229.46	5.4357
	28	202.03	5.3084
Statistic Value	Zero Order	First Order	
r <sup>2</sup>	0.9894	0.9954	
V.R.	656.0465	1503.2800	
d.f.	1,7	1,7	
reject Ho with P	0	0	
rate constant (k)*	-9.3981	-2.9598 E-02	
std.error of k(Sb)	±0.3669	±7.6337 E-04	
intercept.	457.9663	6.1673	

\* The rate constant unit for zero order was mg% / day and for first order was (day<sup>-1</sup>).

Table 20 Degradation data of CPC eye drops BPC containing 15% PF<sub>407</sub> at 60°C.

Formulation	Time (day)	CPC Concentration (mg/100 ml)	ln Concentration
15% PF <sub>407</sub> 60°C	0	475.37	6.1641
	3	439.86	6.0864
	6	392.17	5.9717
	10	361.33	5.8898
	13	336.00	5.8171
	17	298.30	5.6981
	21	281.42	5.6398
	24	249.88	5.5210
	27	229.45	5.4357
Statistic Value	Zero Order	First Order	
r <sup>2</sup>	0.9837	0.9952	
V.R.	422.6325	1444.0750	
d.f.	1,7	1,7	
reject H <sub>0</sub> with P	0	0	
rate constant (k)*	-8.8113	-2.6221 E-02	
std.error of k(Sb)	+0.4286	+6.9001 E-04	
	458.8833	6.1552	

\* The rate constant unit for zero order was mg% / day and for first order was (day<sup>-1</sup>).

could be concluded that the degradation of all formulations were more fitted to the first order than the zero order. The two reasons were

1.  $r^2$  (coefficient of determination) of the first order was closer to 1 than of the zero order, thus the correlation was better.

2. Reject  $H_0$  (null hypothesis) with lesser P (probability) occurred to the first order than to the zero order, thus it was more fitted to the first order more than to the zero order.

The other method determining relationship between concentration and time was by plotting concentration versus time as illustrated in Figures 11-13 and  $\ln$  concentration versus time as in Figures 14-16. The lines of  $\ln$  concentration versus time were more straight than of concentration - time. This result emphasized the aforementioned conclusion.

### 3.3.2 Comparison of Rate Constant

Table 21 and Figure 17 showed the comparison of rate constants and  $r^2$ s of sixteen formulations. All  $r^2$ s are greater than 0.99, which means high correlation.

Five formulations were chosen because of their low rate constants. They were 20% PEG<sub>6000</sub> ( $k = -3.4784$ ), 25% PEG<sub>6000</sub> ( $k = -3.3615$ ), 25% PEG<sub>20000</sub> ( $k = -2.9756$ ), 30% PEG<sub>20000</sub> ( $k = -2.9598$ ) and 15% PF<sub>407</sub> ( $k = -2.6221$ ). These five formulations and standard formulation were incubated at 40°, 50°, 50°C for further study.

### 3.4 Comparison of Physical Properties

Both BPC eye drops of pH 6.0 and 7.0 were similar in tonicity and viscosity. Addition of polymers affected the viscosity and



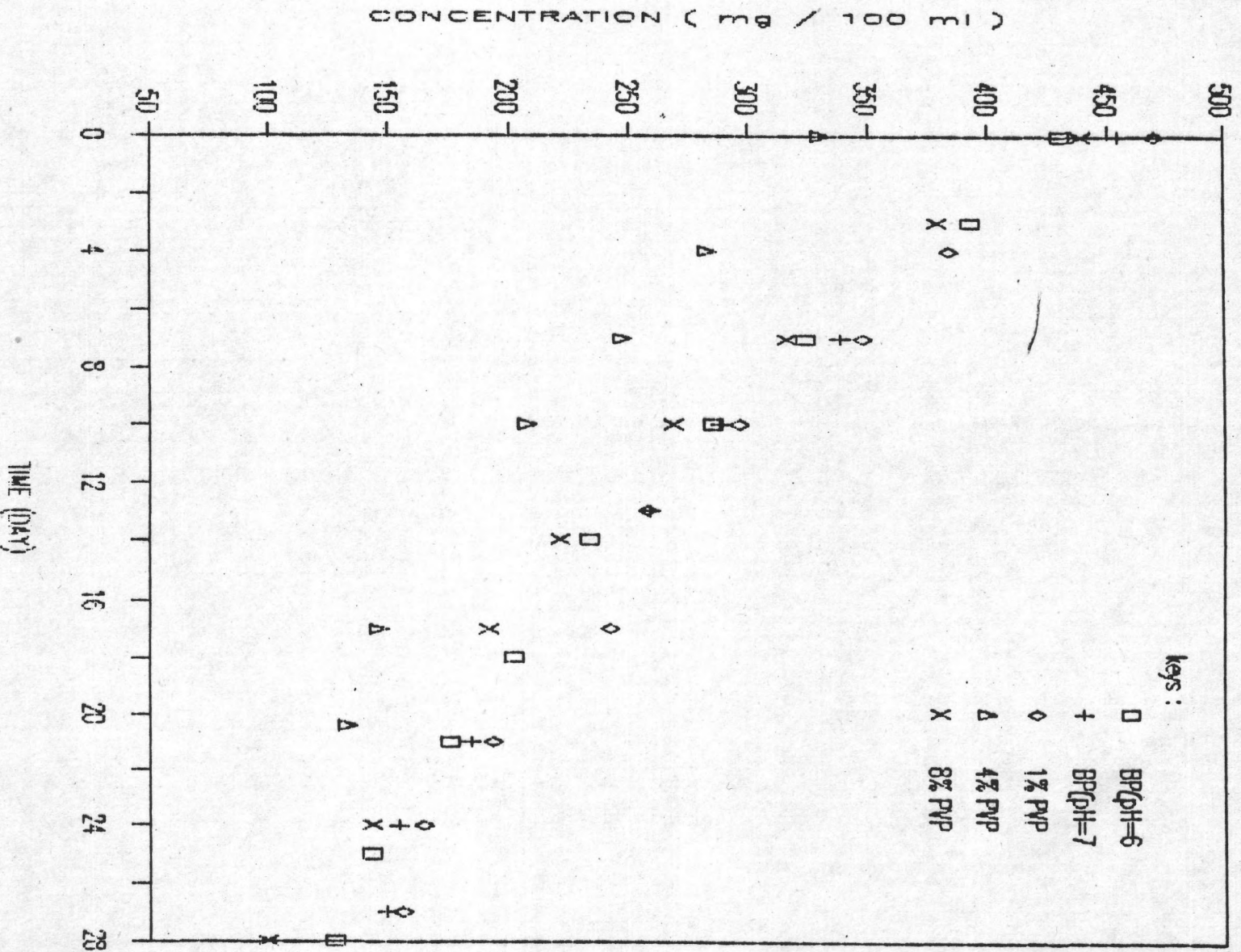


Figure 11 Zero ordered degradation profiles of CPC eye drops  
 BPC pH=6.0, BPC pH=7.0, containing 1%PVP, 4%PVP, 8%PVP at 60°C.

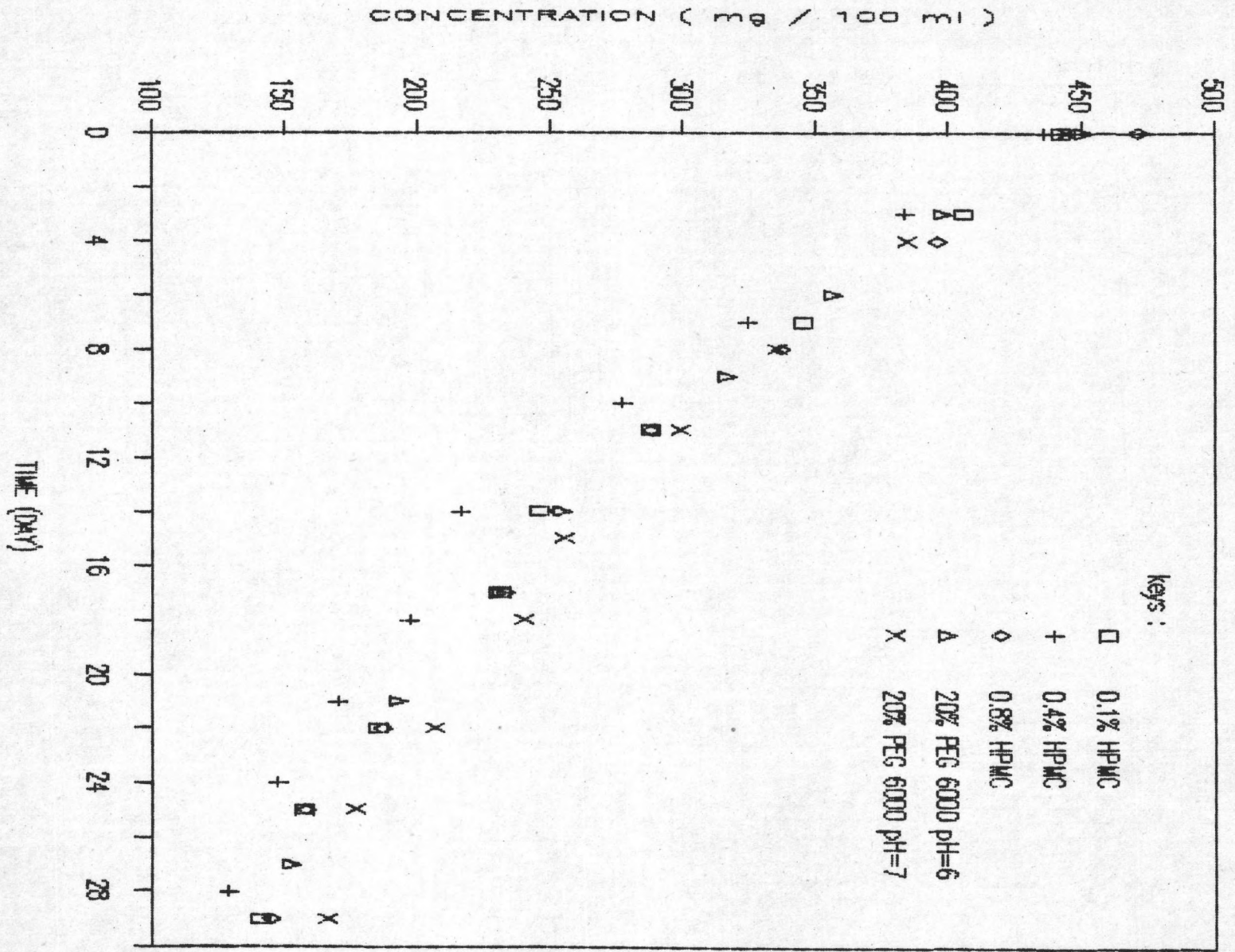


Figure 12 Zero ordered degradation profiles of CPC eye drops containing 0.1%HPWC, 0.4%HPWC, 0.8%HPWC, 20%PEG<sub>6000</sub> pH=6.0, 20%PEG<sub>6000</sub> pH=7.0 at 60°C.

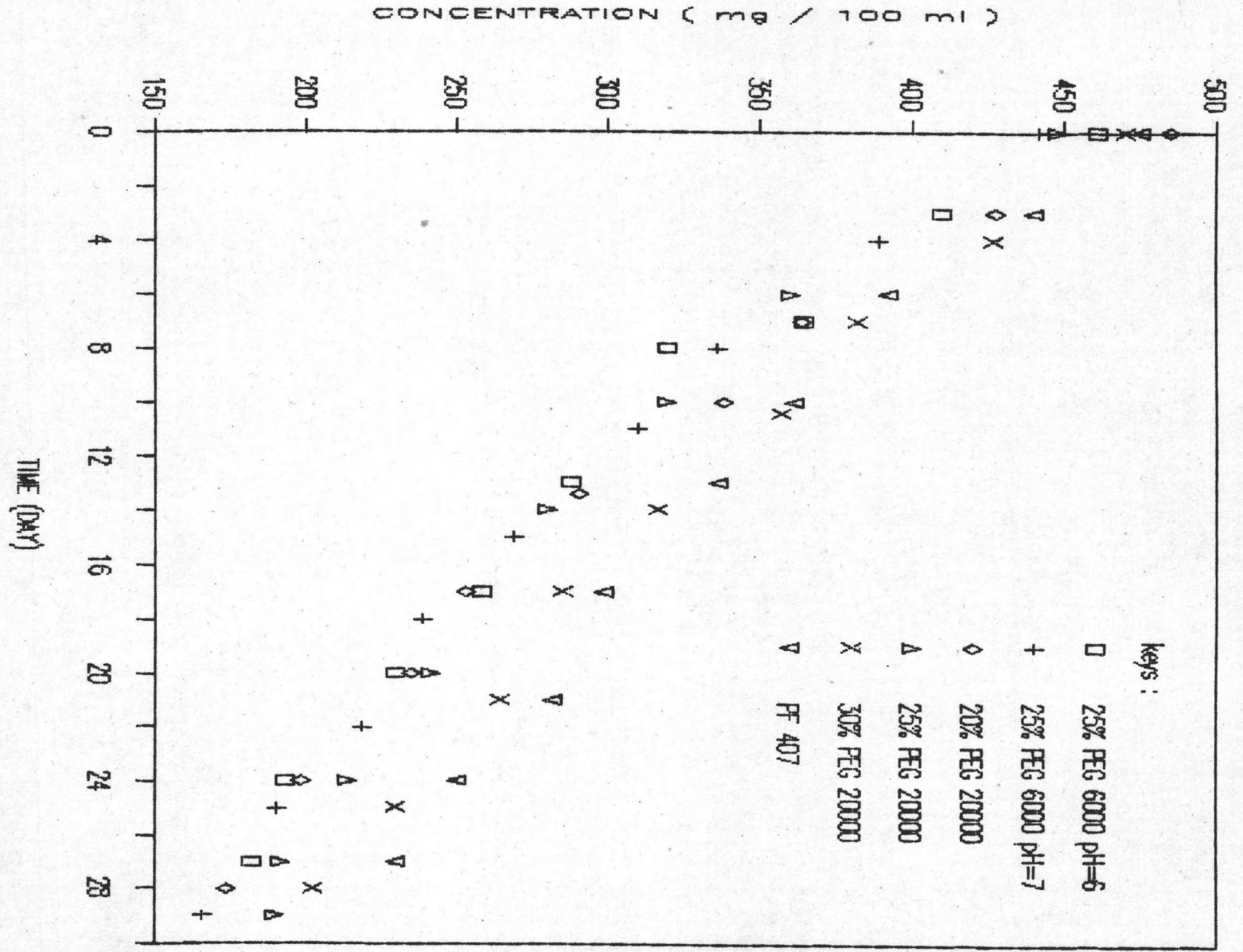


Figure 13 Zero ordered degradation profiles of CPC eye drops containing 25%PEG<sub>6000</sub> pH=6.0, 25%PEG<sub>6000</sub> pH=7.0, 20%PEG<sub>20000</sub>, 25%PEG<sub>20000</sub>, 30%PEG<sub>20000</sub>, 15%PF<sub>407</sub> at 60°C.



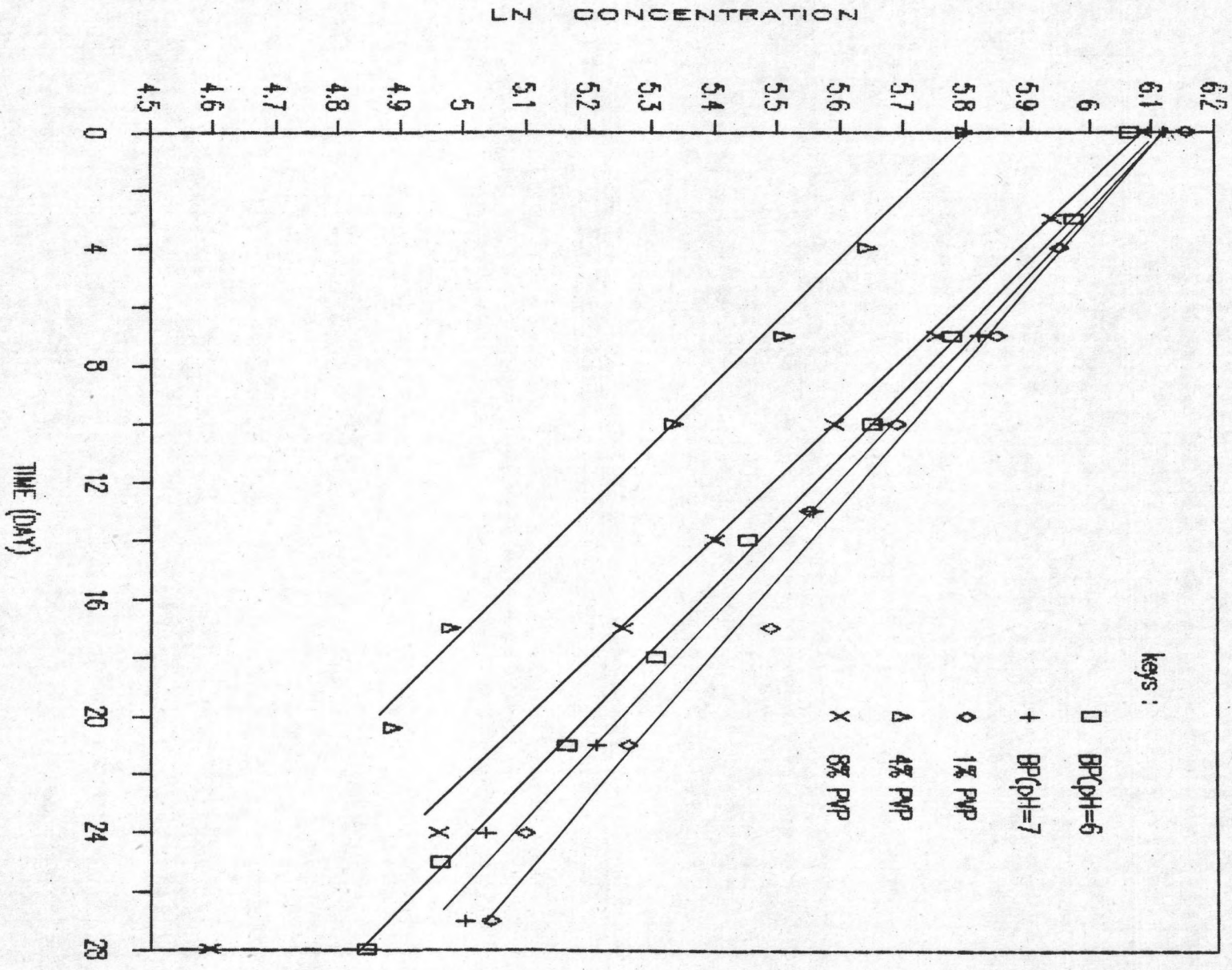


Figure 14 First ordered degradation profiles of CPC eye drops  
BPC pH=6.0, BPC pH=7.0, containing 1%PVP, 4%PVP, 8%PVP at 60°C.



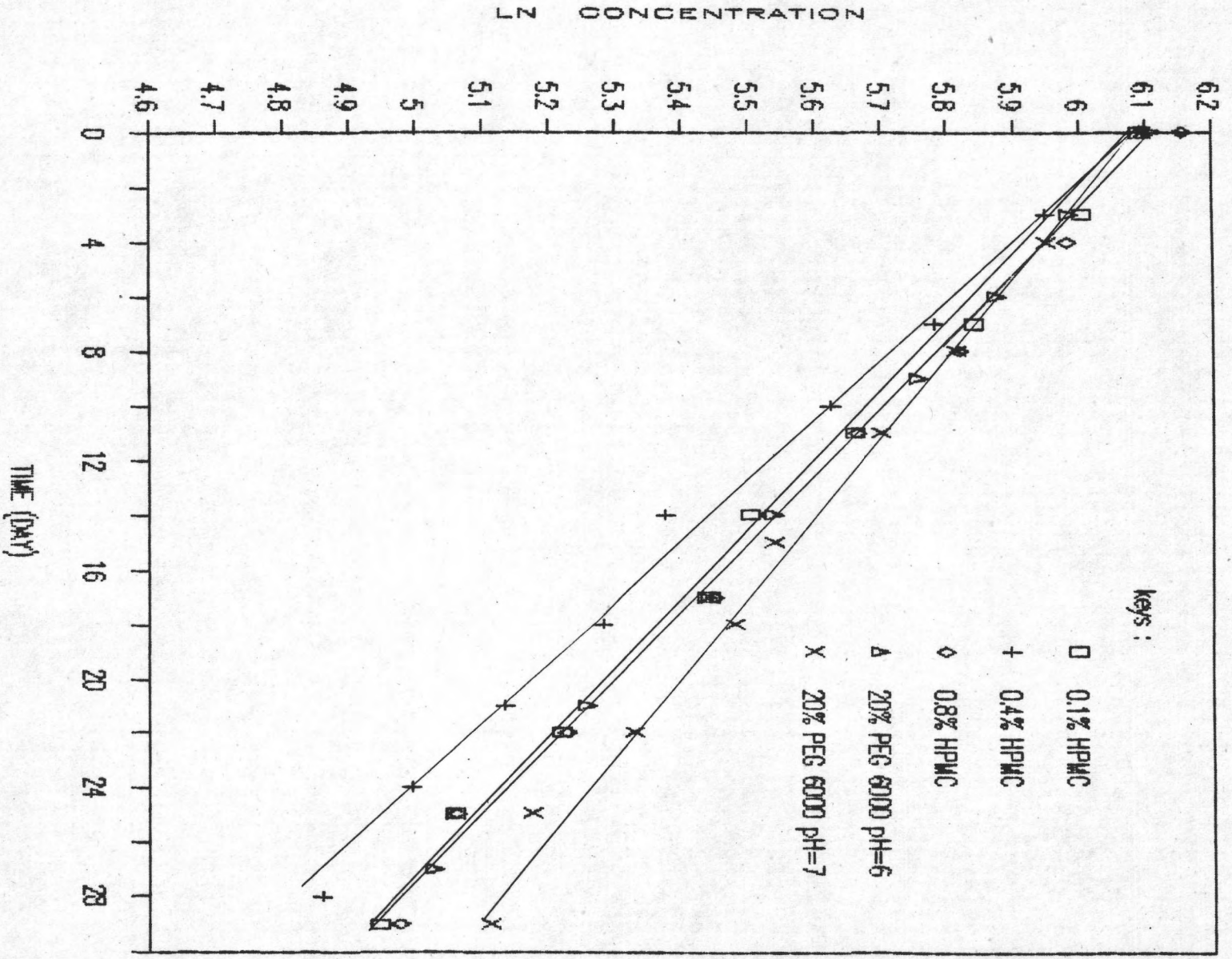


Figure 15 First ordered degradation profiles of CPC eye drops containing 0.1%HPMC, 0.4%HPMC, 0.8%HPMC, 20%PEG<sub>6000</sub> pH=6.0, 20%PEG<sub>6000</sub> pH=7.0 at 60°C.

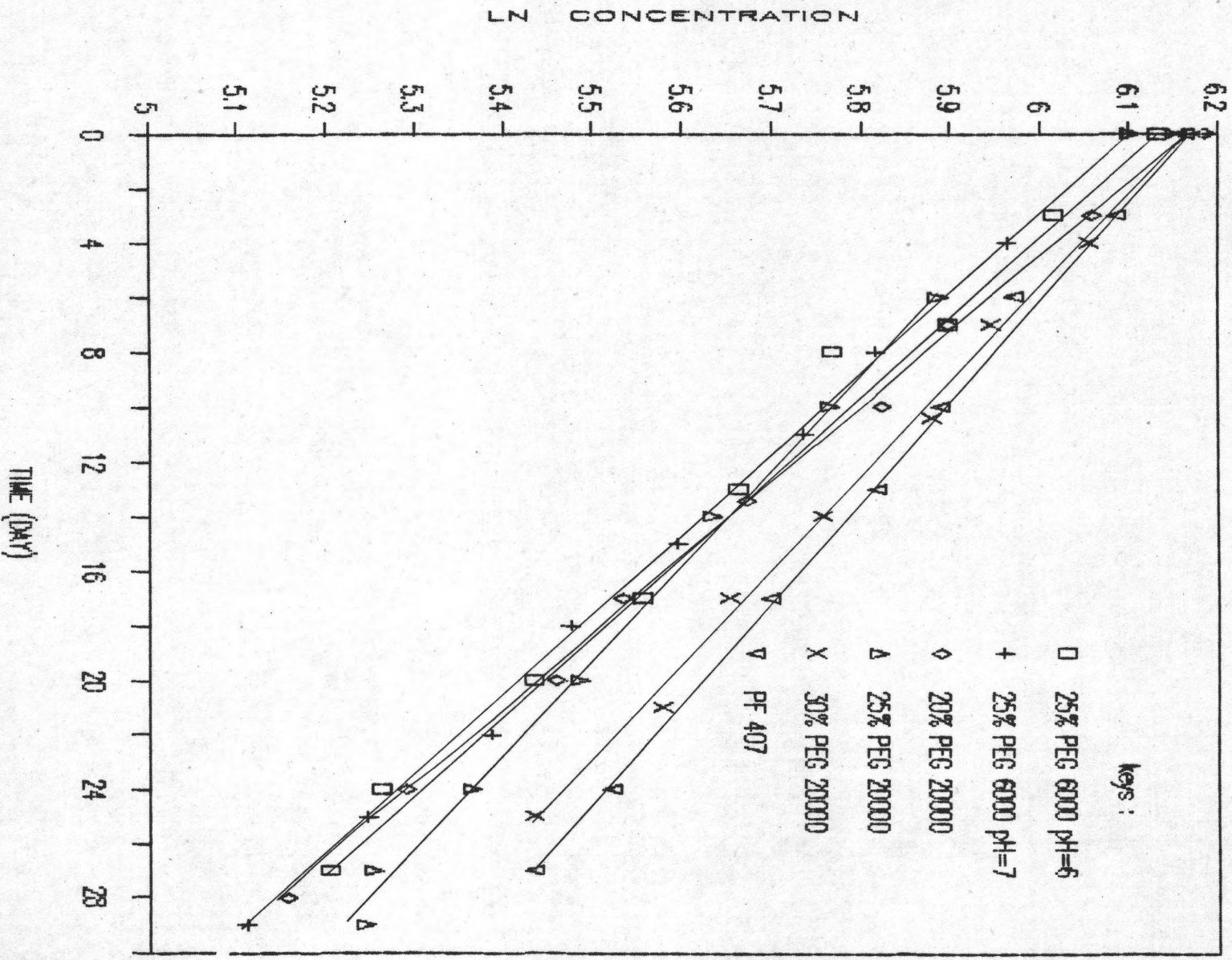


Figure 16 First ordered degradation profiles of CPC eye drops containing 25%PEG<sub>6000</sub> pH=6.0, 25%PEG<sub>6000</sub> pH=7.0, 20%PEG<sub>20000</sub>, 25%PEG<sub>20000</sub>, 30%PEG<sub>20000</sub>, 15%PF<sub>407</sub> at 60°C.

Table 21 Comparison of rate constants of sixteen feasible CPC eye drops at 60°C.

Formulations		Rate Constant (k) X10 <sup>-2</sup> (day <sup>-1</sup> )	Std. error of k X10 <sup>-4</sup>	95% Confidence Interval of Rate Constant X10 <sup>-2</sup> (day <sup>-1</sup> )	Coefficient of Determination (r <sup>2</sup> )
1	BPC	-4.2770	13.5263	-4.53-(-4.02)	0.9950
2	HPC pH = 6.0	-4.4300	5.9200	-4.67-(-4.19)	0.9988
3	1% PVP	-4.1239	14.0941	-4.36-(-3.89)	0.9919
4	4% PVP	-4.6849	17.3792	-5.24-(-4.12)	0.9945
5	8% PVP	-5.0877	18.5695	-5.58-(-4.60)	0.9921
6	0.1% HPMC	-4.0718	8.8611	-4.31-(-3.84)	0.9967
7	0.4% HPMC	-4.4346	11.9208	-4.67-(-4.20)	0.9950
8	0.8% HPMC	-4.1576	9.9418	-4.39-(-3.92)	0.9960
9	20% PEG <sub>6000</sub> *	-3.4784	8.7778	-3.24-(-3.71)	0.9956
10	20% PEG <sub>6000</sub> pH=6.0	-4.0167	3.4119	-4.02-(-4.06)	0.9996
11	25% PEG <sub>6000</sub> *	-3.3615	5.4540	-3.12-(-3.59)	0.9982
12	25% PEG <sub>6000</sub> pH=6.0	-3.4882	6.4947	-3.72-(3.25)	0.9976
13	20% PEG <sub>20000</sub>	-3.6494	5.7646	-3.89-(-3.41)	0.9983
14	25% PEG <sub>20000</sub> *	-2.9756	8.0970	-2.73-(-3.22)	0.9956
15	30% PEG <sub>20000</sub> *	-2.9598	7.6337	-2.72-(-3.19)	0.9954
16	15% PF <sub>407</sub> *	-2.6221	6.9001	-2.38-(-2.85)	0.9952

\* selected formulations of low rate constants.



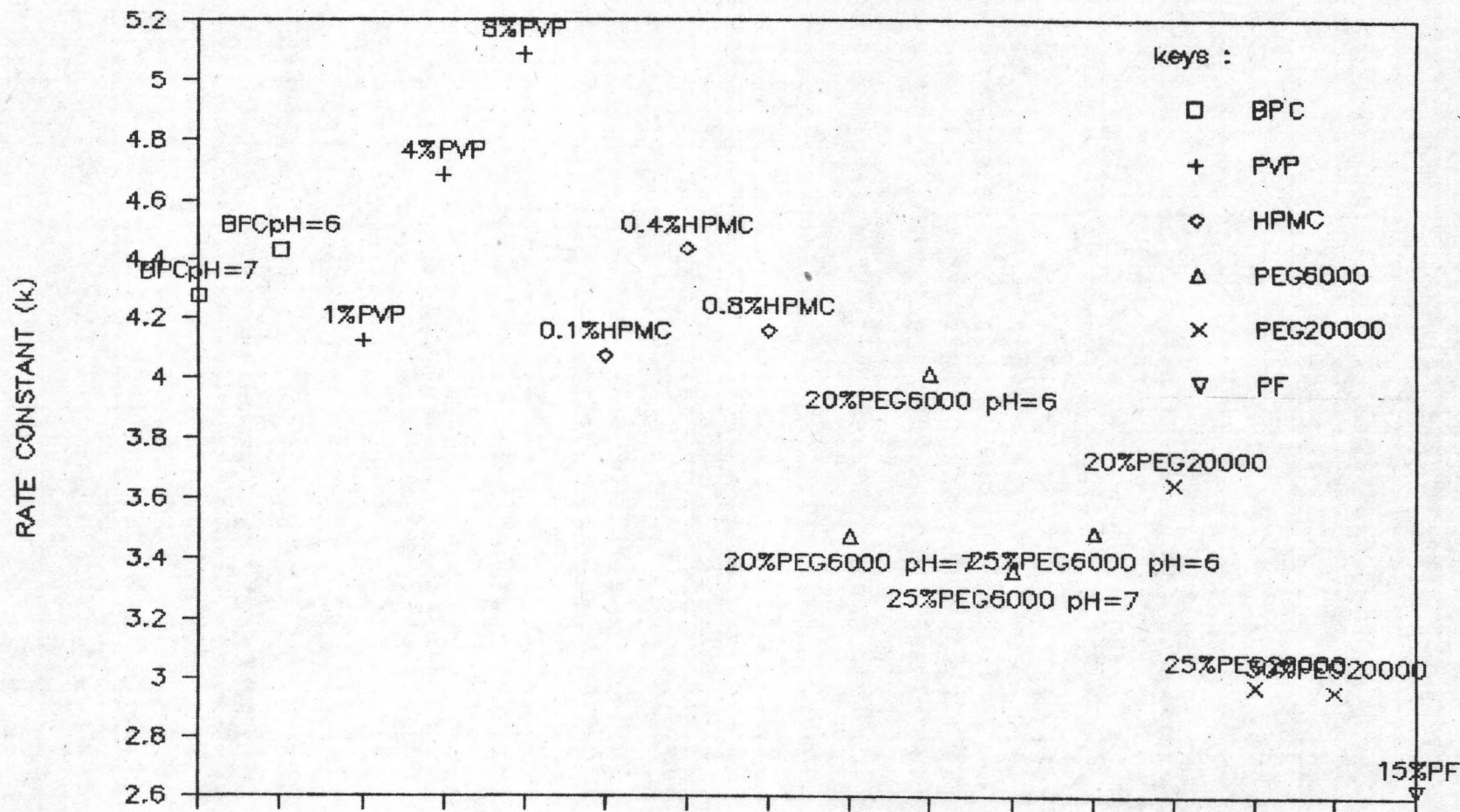


Figure 17 Comparison of rate constants(k) of sixteen CPC eye drops formulations at 60°C.



tonicity of the eye drops as shown in Table 22. Both PVP and HPMC markedly increased the viscosity but slightly affected the tonicity while PEG's extensively increased both properties, and PF<sub>407</sub> moderately increased the properties.

After incubation at 60°C, the pH's of all eye drops were slightly dropped. However, they were within the tolerant limit of 5-9 except the PEG formulations which pH's were initially adjusted to 6.0, then dropped to about 3.

Most formulations exhibited clear white solution after preparation. The formulations of 4% and 8% PVP showed slightly clear yellow solution after preparation. After incubation, both BPC eye drops were observed discoloration. So did the formulation containing HPMC especially at lower concentration. A yellow color appeared in formulation containing PVP, PEG and PF<sub>407</sub>. Increasing the concentration of PVP and PEG and PF<sub>407</sub> darkened the color.

### 3.5 Stability at 40°, 50°, 55°, 60° C

The six formulations (BPC, 20%PEG<sub>6000</sub>, 25%PEG<sub>6000</sub>, 25%PEG<sub>20000</sub>, 30%PEG<sub>20000</sub>, 15%PF<sub>407</sub>) were chosen to be incubated at 40°, 50° and 55° C. Tables 23-28 exhibited four temperatures degradation data such as rate constant (k), intercept,  $r^2$ , variance ratio (F), degree of freedom (d.f.), standard error of slope (Sb) and probability (P) that rejected null hypothesis ( $H_0$ ). Figures 18-23 showed the first order degradation profiles of CPC eye drops at various temperatures. Most of the relation were fitted with first order as described in 60° C incubation (except 20%PEG<sub>6000</sub> at 40° C, 25%PEG<sub>6000</sub>

Table 22 Comparison of physical properties\* of CPC eye drops before and after incubation at 60°C for 1 month.

Formulations	Tonicity (milliosmoles)	Viscosity ( $\eta$ )		pH		Discoloration	
		centistoke	cps.	Before 60°C Incubation	After 60°C Incubation	Before 60°C Incubation	After 60°C Incubation
BPC pH = 7.0*	263.5	0.93	0.94	7.06	6.84	0	0
BPC pH = 6.0	259.5	0.93	0.94	5.95	5.54	0	0
1%PVP	274.5	3.40	3.43	7.13	6.80	0	+
4%PVP	284.5	28.70	29.18	7.08	6.55	+	++
8%PVP	296.0	174.0	177.84	7.08	6.69	++	++++
0.1%HPMC	276.0	1.50	1.51	7.01	6.77	0	0
0.4%HPMC	280.5	6.00	6.06	7.04	6.73	0	0
0.8%HPMC	282.5	23.90	24.15	7.07	6.74	0	+
20%PEG <sub>8000</sub> pH=7*	704.0*	15.40	16.02	7.27	6.40	0	++
20%PEG <sub>8000</sub> pH=6	701.0*	15.40	16.02	5.82	3.70*	0	++
25%PEG <sub>8000</sub> pH=7*	1067.0*	25.90	27.18	7.27	6.35	0	+++
25%PEG <sub>8000</sub> pH=6	1060.0*	25.90	27.18	6.06	3.85*	0	+++
20%PEG <sub>20000</sub>	707.5*	58.80	61.25	7.38	6.90	0	++
25%PEG <sub>20000</sub> *	1076.0*	118.00	123.88	7.51	7.10	0	+++
30%PEG <sub>20000</sub> *	unmeasurable	213.00	225.30	7.51	7.38	0	++++
15%PF <sub>407</sub> *	474.0	24.00	24.57	7.22	6.93	0	+++

\* the formulation for further study

# higher tolerance limit of the eyes

o clear white

+ clear yellow

Table 23 Four temperatures degradation data of CPC eye drops BPC 1973.

Formulation	40°C			50°C			55°C			60°C		
	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.
BPC	0	486.09	6.1864	0	473.68	6.1605	0	475.46	6.1643	0	454.52	6.1192
	13	465.87	6.1439	7	427.11	6.0570	3	442.18	6.0917	7	338.34	5.8241
	26	429.59	6.0558	14	381.99	5.9454	9	380.95	5.9427	10	288.04	5.6631
	39	398.76	5.9884	21	341.47	5.8333	13	340.38	5.8300	13	259.08	5.5571
	52	371.13	5.9166	28	298.31	5.6981	17.27	299.56	5.7023	21	183.63	5.2129
	67	353.33	5.8674	36	273.60	5.6117	22	265.23	5.5806	24	153.76	5.0354
	94	305.31	5.7213	42	248.66	5.5161	27	237.64	5.4707	27	148.94	5.0035
				49	217.79	5.3835						
Statistic value	Zero Order	First Order	Zero Order	First Order	Zero Order	First Order	Zero Order	First Order	Zero Order	First Order		
$r^2$	0.9882	0.9952	0.9863	0.9978	0.9889	0.9987	0.9636	0.9950				
V.R.	418.1367	1036.7240	430.6794	2754.4710	441.5095	3762.4060	132.1967	999.8200				
d.f.	1,5	1,5	1,6	1,6	1,5	1,5	1,5	1,5				
Reject $H_0$ with P	0.0001	0.0001	0	0	0.0001	0	0.0004	0.0001				
Rate constant (k)*	-1.9643	-5.0102 E-03	-5.1640	-1.5695E-02	-9.0096	-2.6253E-02	-11.1254	-4.2770E-02				
std.error of k(Sb)	$\pm 9.6062$ E-02	$\pm 1.5560$ E-04	$\pm 0.2488$	$\pm 2.9905$ E-04	$\pm 0.4288$	$\pm 4.2801$ E-04	$\pm 0.9676$	$\pm 1.3526$ E-03				
intercept.	483.0997	6.1911	459.9908	6.1622	466.2443	6.1683	423.0137	6.1111				

The rate constant unit for zero order was  $\text{mg}^* / \text{day}$  and for first order was  $(\text{day}^{-1})$ .



Table 24 Four temperatures degradation data of CPC eye drop BPC 1973 with 20% PEG<sub>6000</sub>.

Formulation	40°C			50°C			55°C			60°C		
	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.
20% PEG <sub>6000</sub>	0	488.19	6.1922	0	481.78	6.1775	0	471.06	6.1550	0	444.43	6.0968
	14	458.04	6.1269	7	434.00	6.0730	3	445.74	6.0997	4	384.19	5.9511
	24	441.50	6.0902	14	390.38	5.9671	9	392.85	5.9734	8	335.70	5.8162
	42	412.30	6.0211	21	360.86	5.8885	13	354.48	5.8707	11	299.42	5.7018
	59	387.02	5.9585	28	327.46	5.7914	17.27	325.86	5.7865	15	254.76	5.5403
	70	354.55	5.8708	36	285.37	5.6538	22	296.61	5.6924	18	240.11	5.4811
	88	334.64	5.813				27	268.92	5.5944	22	206.63	5.3309
										25	177.18	5.1772
									29	166.29	5.1137	
Statistic value	Zero Order		First Order	Zero Order		First Order	Zero Order		First Order	Zero Order		First Order
r <sup>2</sup>	0.9932		0.9919	0.9934		0.9971	0.9900		0.9988	0.9723		0.9956
V.R.	732.3920		610.5413	602.6553		1378.2970	493.1490		4143.6460	245.8943		1570.3090
d.f.	1,5		1,5	1,4		1,4	1,5		1,5	1,7		1,7
Reject H <sub>0</sub> with P	0.0001		0.0001	0.0004		0.0002	0.0001		0	0		0
rate constant (k)*	-1.7558		-4.3245E-03	-5.3214		-1.4181E-02	-7.6270		-2.1082E-02	-9.6038		-3.4784E-02
std.error of k(Sb)	±6.4878 E-02		±1.7502E-04	±0.2168		±3.8197E-04	±0.3435		±3.2750E-04	±0.6214		±8.7778E-04
intercept.	485.4493		6.1939	473.9862		6.1757	464.5195		6.1566	419.6009		6.0890



The rate constant unit for zero order was mg% / day and for first order was (day<sup>-1</sup>).



Table 25 Four temperatures degradation data of CPC eye drops BPC 1973 containing 25% PEG<sub>6000</sub>.

Formulation	40°C			50°C			55°C			60°C		
	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.
25% PEG <sub>6000</sub>	0	481.01	6.1759	0	431.89	6.0682	0	481.19	6.1763	0	441.85	6.0910
	14	467.51	6.1474	7	392.45	5.9726	3	455.61	6.1216	4	389.07	5.9638
	28	436.93	6.0798	14	352.84	5.8660	9	400.49	5.9926	8	335.84	5.8166
	42	421.33	6.0434	21	321.57	5.7732	13	372.14	5.9193	11	309.55	5.7351
	59	390.95	5.9686	29	301.22	5.7078	17.27	341.09	5.8321	15	269.03	5.5948
	88	349.85	5.8575	35	270.51	5.6003	22	300.62	5.7058	18	238.54	5.4745
				42	247.41	5.5110	27	264.45	5.5776	22	218.42	5.3864
										25	190.01	5.2471
										29	165.62	5.1097
	Statistic value	Zero Order	First Order	Zero Order	First Order	Zero Order	First Order	Zero Order	First Order	Zero Order	First Order	
	r <sup>2</sup>	0.9950	0.9938	0.9872	0.9955	0.9985	0.9955	0.9819	0.9982			
	V.R.	788.1639	638.447	385.7912	1118.4270	3331.6350	1118.426	378.8621	3798.8			
d.f.	1,4	1,4	1,5	1,5	1,5	1,5	1,7	1,7				
Reject H <sub>0</sub> with P	0.0003	0.0003	0.0001	0.0001	0	0.0001	0	0				
rate constant (k)*	-1.5277	-3.6975 E-03	-4.3074	-1.3050E-02	-8.0143	-2.1941E-02	-9.3987	-3.3615E-02				
std.error of k(Sb)	+5.4417 E-02	+1.4634 E-04	+0.2193	+3.9022E-04	+0.1388	+6.5607E-02	+0.4829	+5.4540E-04				
intercept.	483.4139	6.1878	422.2109	6.0615	478.1509	6.1897	422.0623	6.0951				

The rate constant unit for zero order was mg% / day and for first order was (day<sup>-1</sup>).

Table 26 Four temperatures degradation data of CPC eye drops BPC 1973 containing 25% PEG<sub>20000</sub>.

Formulation	40°C			50°C			55°C			60°C		
	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.
25% PEG <sub>20000</sub>	13.25	449.16	6.1074	7	453.41	6.1168	0	484.52	6.1832	0	447.45	6.1036
	26	424.89	6.0518	28	349.57	5.8567	3	450.95	6.1114	6.0208	360.31	5.8870
	42	402.38	5.9974	42	292.34	5.6779	9	407.90	6.0110	10	319.15	5.7657
	60	378.43	5.9360	49	264.06	5.5761	13	374.01	5.9243	14	279.54	5.6331
	74	357.09	5.8780	56	240.25	5.4817	17.27	344.15	5.8411	20	241.09	5.4852
				63	224.54	5.4140	22	323.39	5.7789	24	213.53	5.3638
							27	286.29	5.6574	27	191.22	5.2534
										29	189.28	5.2432
Statistic value	Zero Order	First Order	Zero Order	First Order	Zero Order	First Order	Zero Order	First Order	Zero Order	First Order		
r <sup>2</sup>	0.9965	0.9981	0.9896	0.9990	0.9908	0.9968	0.9682	0.9956				
V.R.	857.5114	1595.3570	379.2753	3883.7450	539.0433	1560.5490	182.6472	1350.5180				
d.f.	1,3	1,3	1,4	1,4	1,5	1,5	1,6	1,6				
Reject H <sub>0</sub> with P	0.0001	0.0001	0.0005	0.0002	0.0001	0.0001	0.0001	0				
Rate constant (k)*	-1.4785	-3.6872 E-03	-4.1489	-1.2769E-02	-7.1583	-1.8933 E-02	-8.5722	-2.9756E-02				
std.error of k(Sb)	+5.0491 E-02	+9.2314 E-05	+0.2130	+2.0490E-04	+0.3083	+4.7927 E-04	+0.6343	+8.0970E-04				
intercept.	466.0409	6.1529	473.4414	6.2086	474.9497	6.1765	419.5165	6.0755				

The rate constant unit for zero order was mg% / day and for first order was (day<sup>-1</sup>).



Table 27 Four temperatures degradation data of CPC eye drops BPC 1973 containing 30% PEG<sub>20000</sub>.

Formulation	40°C			50°C			55°C			60°C		
	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.
30%PEG <sub>20000</sub>	0	470.75	6.1543	0	481.23	6.1763	0	487.02	6.1883	0	470.32	6.1543
	13.25	448.44	6.1058	3	474.32	6.1619	4	448.03	6.1049	4	426.83	6.0546
	26	425.38	6.0530	10	459.27	6.1296	8.27	408.40	6.0122	7	382.02	5.9455
	42	411.11	6.0189	17	423.48	6.0485	13	380.86	5.9424	10.4167	357.52	5.8792
	60	367.50	5.9067	24	388.14	5.9614	18	340.06	5.8291	14	316.37	5.7569
	74	351.56	5.8624	32	366.13	5.9030	25	324.13	5.7811	17	284.81	5.6518
				38	335.30	5.815	26	307.72	5.7292	21	264.26	5.5769
										25	229.46	5.4357
										28	202.03	5.3084
	Statistic value	Zero Order	First Order	Zero Order	First Order	Zero Order	First Order	Zero Order	First Order	Zero Order	First Order	
r <sup>2</sup>	0.9888	0.9853	0.9885	0.9828	0.9803	0.9923	0.9894	0.9954				
V.R.	353.0386	267.4075	472.9684	286.5271	249.4382	644.3046	656.0465	1503.28				
d.f.	1,4	1,4	1,5	1,5	1,5	1,5	1,7	1,7				
Reject H <sub>0</sub> with P	0.0005	0.0006	0.0001	0.0002	0.0002	0.0001	0	0				
rate constant (k)*	-1.6243	-3.9889 E-03	-3.9162	-9.5717 E-03	-6.8793	-1.7791 E-02	-9.3981	-2.9598 E-0				
std error of k(Sb)	±8.6447 E-02	±2.4393 E-04	±0.1893	±5.6547 E-04	±0.4356	±7.0090 E-04	±0.3669	±7.6337 E-04				
intercept.	470.7277	6.1599	487.6404	6.1975	475.1169	6.1736	457.9663	6.1673				

The rate constant unit for zero order was mg% / day and for first order was (day<sup>-1</sup>).

Table 28 Four temperatures degradation data of CPC eye drops BPC 1973 containing 15% PF<sub>407</sub>.

Formulation	40°C			50°C			55°C			60°C		
	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.	Time (day)	CPC Conc. (mg/100 ml)	ln Conc.
15% PF <sub>407</sub>	0	480.23	6.1743	7	451.79	6.1132	0	468.00	6.1485	0	475.37	6.1641
	14	459.56	6.1303	14	419.60	6.0393	3	434.77	6.0748	3	439.86	6.0864
	25	433.23	6.0713	21	393.76	5.9757	7	404.34	6.0023	6	392.17	5.9717
	38	416.57	6.0321	29	365.78	5.9020	11.27	369.25	5.9115	10	361.33	5.8898
	58	402.11	5.9967	35	348.13	5.8526	16	340.42	5.8302	13	336.00	5.8171
	74	371.79	5.9183	42	323.40	5.7789	25.25	295.63	5.6891	17	298.30	5.6981
	92	349.99	5.8579				29	279.53	5.6331	21	281.42	5.6398
	106.028	338.13	5.8234							24	249.88	5.5210
	119	321.73	5.7739							27	229.45	5.4357
Statistic value	Zero Order	First Order	Zero Order	First Order	Zero Order	First Order	Zero Order	First Order	Zero Order	First Order		
r <sup>2</sup>	0.9896	0.9941	0.9957	0.9990	0.9785	0.9943	0.9837	0.9952				
V.R.	668.6757	1180.2540	919.8505	3811.5050	228.0944	866.0086	422.6325	1444.0750				
d.f.	1,7	1,7	1,4	1,4	1,5	1,5	1,7	1,7				
Rejec H <sub>0</sub> with P	0	0	0.0003	0.0002	0.0002	0.0001	0	0				
rate constant (k)*	-1.3065	-3.3150 E-03	-3.5976	-9.3723 E-03	-6.3261	-1.7530 E-02	-8.811318	-2.6221 E-02				
std.error of k(Sb)	+5.0525 E-02	+9.6494 E-05	+0.1186	+1.5181 E-04	+0.4189	+5.9570 E-04	+0.4286	+6.9001 E-04				
interecpt.	473.4007	6.1691	472.4850	6.1748	452.9865	6.1277	458.8833	6.1552				



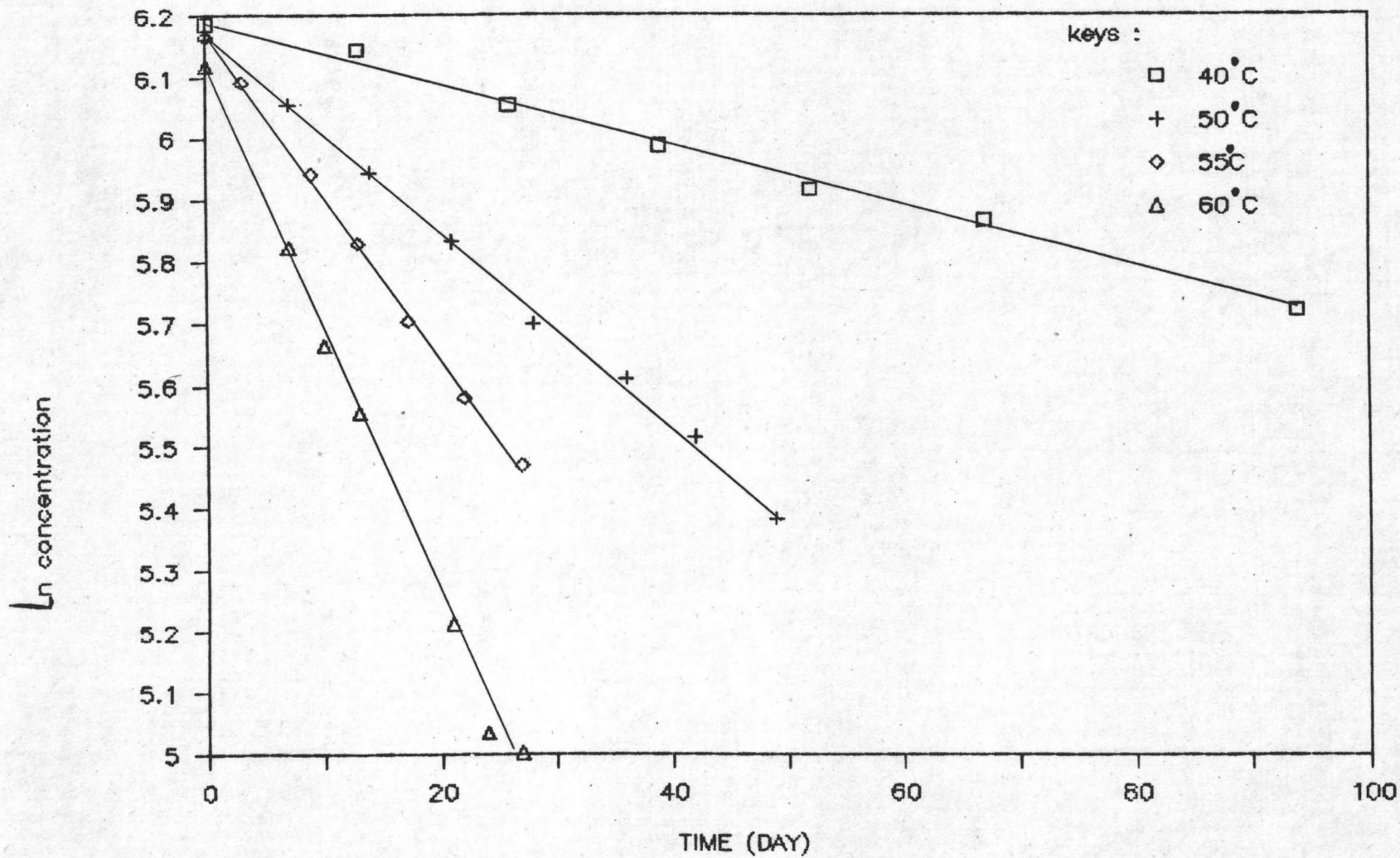


Figure 18 First ordered degradation profiles of CPC eye drops at various temperatures.

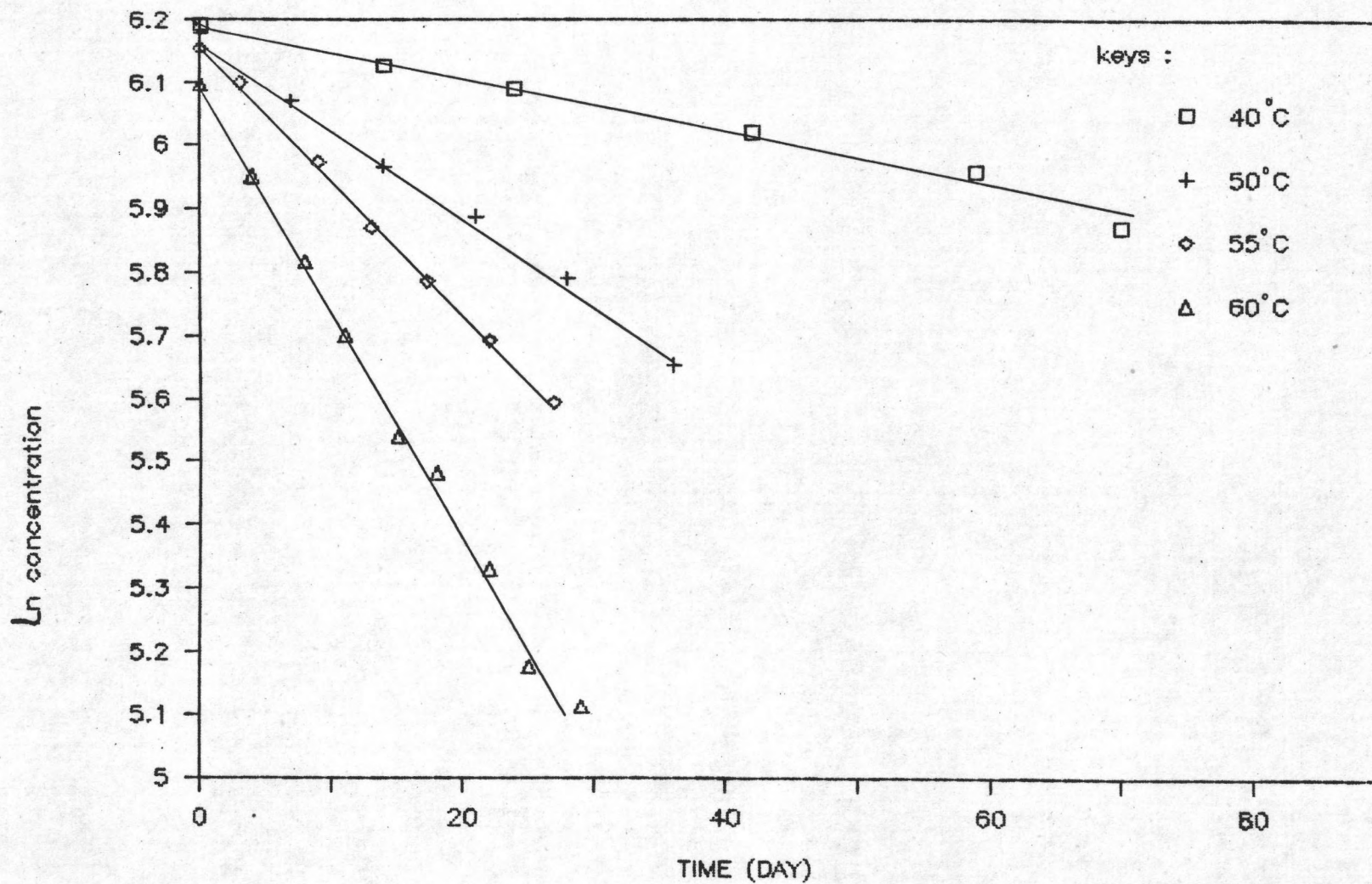


Figure 19 First ordered degradation profiles of CPC eye drops containing 20% PEG<sub>6000</sub> at various temperatures.

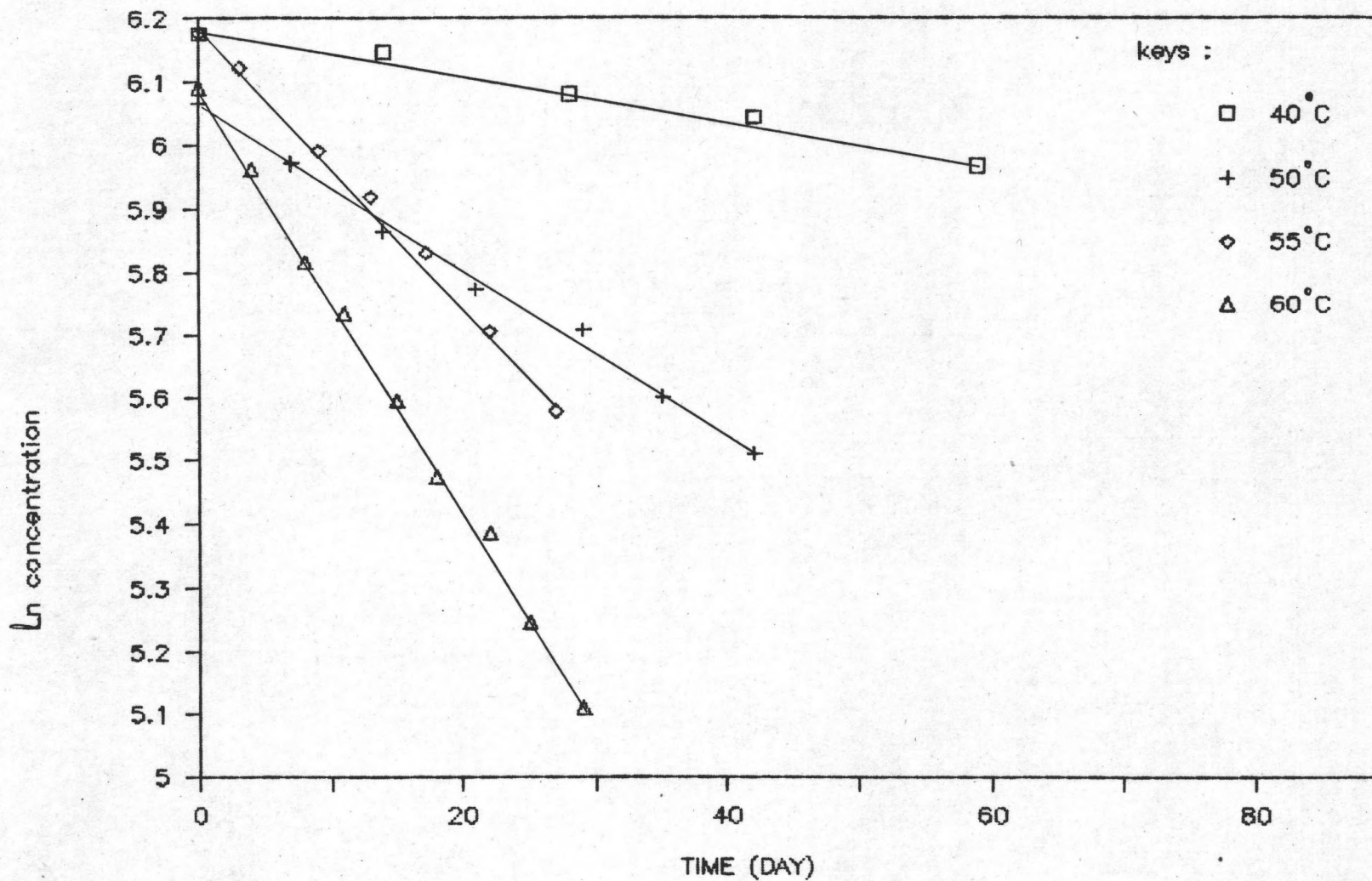


Figure 20 First ordered degradation profiles of CPC eye drops containing 25% PEG<sub>6000</sub> at various temperatures.



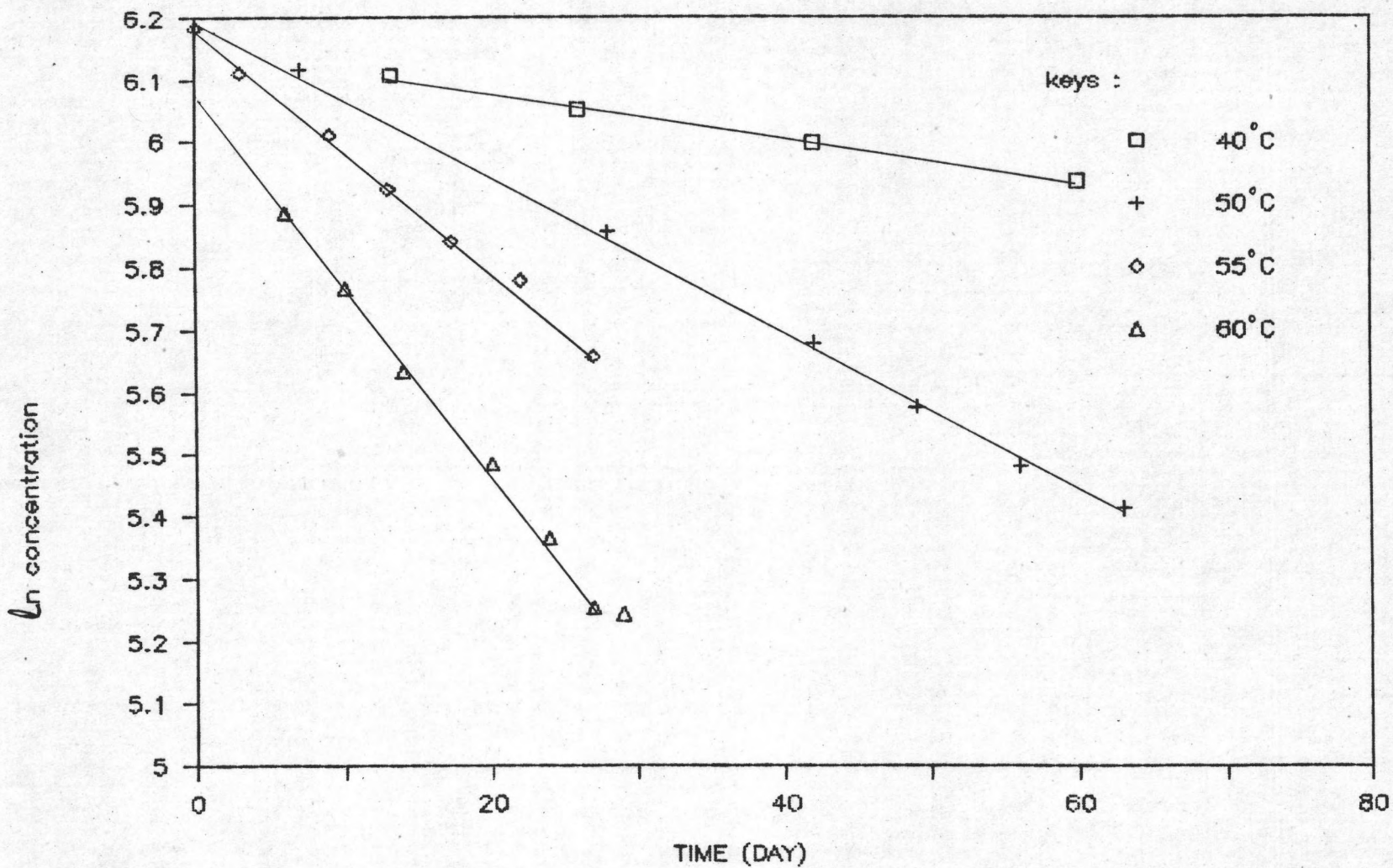


Figure 21 First ordered degradation profiles of CPC eye drops containing 25% PEG<sub>20000</sub> at various temperatures.



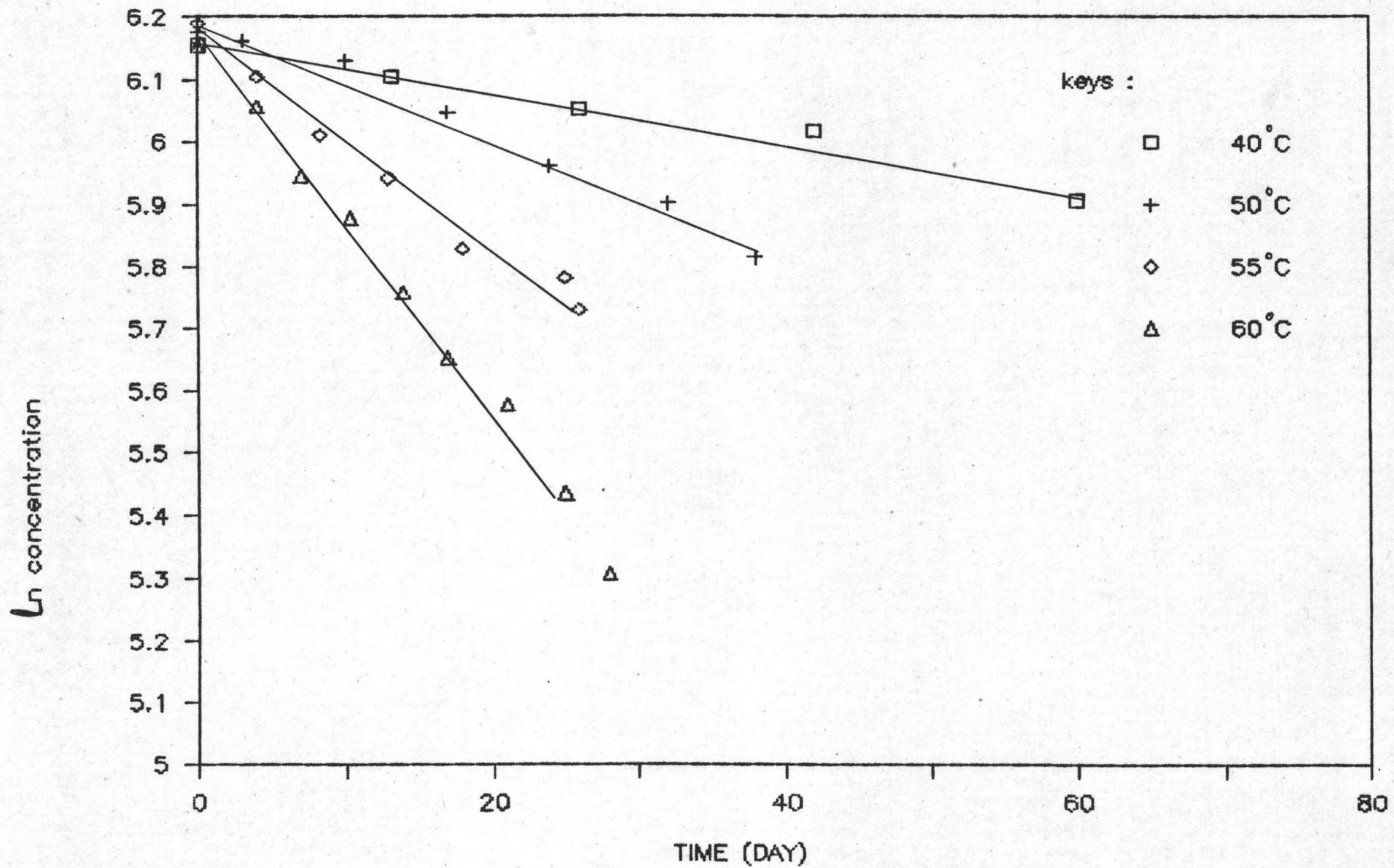


Figure 22 First ordered degradation profiles of CPC eye drops, containing 30% PEG<sub>20000</sub> at various temperatures.

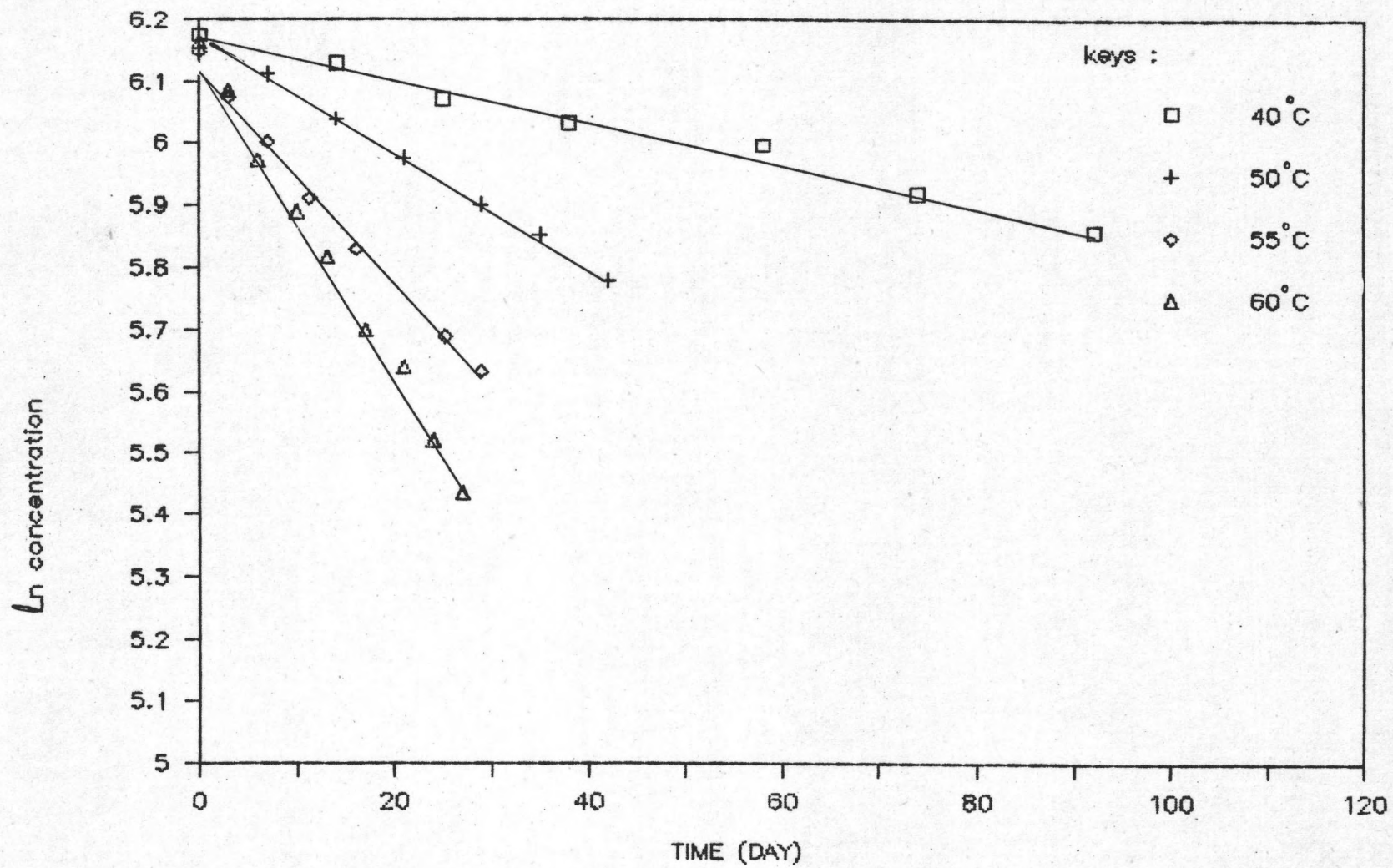


Figure 23 First ordered degradation profiles of CPC eye drops containing 15% PF<sub>407</sub> at various temperatures.



at 40° and 55°C).

All coefficients of determination ( $r^2$ ) were more than 0.99 (except 30%PEG<sub>20000</sub> at 40°C,  $r^2 = 0.9853$  and 50°C,  $r^2 = 0.9828$ ), thus the relationship between ln concentration and time was a straight line.

### 3.6 Arrhenius Plot and Arrhenius Equations

Arrhenius equations and other statistics values  $r^2$ , V.R., d.f.,  $P_0$  calculated by linear regression statistical technique were listed in Tables 29-34.

Comparison of calculated Arrhenius plot of the six selected formulations were shown in Figure 24.

### 3.7 Heat of Activation

From the slope of Arrhenius equation, the heat of activation ( $E_a$ ) was calculated (Appendix D). The comparison of all heat of activation were listed in Table 35. All  $E_a$ 's were between 20 k.cal/mol. to 23 k.cal/mol. The  $E_a$  of the formulation containing 30% PEG<sub>20000</sub> was the lowest (20,801.84 cal/mol.) and the  $E_a$  of the formulation containing 25% PEG<sub>6000</sub> was the highest (23,095.12 cal/mol.).

### 3.8 Calculated Rate Constant and Shelf-life at 25°C and 8°C

Degradation rate constants that were calculated to 25°C and 8°C were compared in Tables 36-37 and Figures 25-26. At 25°C the orderly rank of rate constant in each formulation was shown the following BPC > 20% PEG<sub>6000</sub> > 30% PEG<sub>20000</sub> > 25% PEG<sub>20000</sub> > 25% PEG<sub>6000</sub> > 15% PF<sub>407</sub>. At 8°C the orderly rank of rate constant in each formulation was shown the following 20% PEG<sub>6000</sub> > BPC > 30% PEG<sub>20000</sub> > 25% PEG<sub>20000</sub> > 15% PF<sub>407</sub> > 25% PEG<sub>6000</sub>.



Table 29 Arrhenius equation of CPC eye drops BPC 1973.

Temperature		Degradation rate constant of chloramphenicol			
°C	(1/T) X 10 <sup>3</sup> (kelvin <sup>-1</sup> )	k (day <sup>-1</sup> )	interval of k (day <sup>-1</sup> )	ln k	interval of ln k
60°	3.003	4.2770 E-02	0.0402-0.0453	-3.1519	-3.2138-(-3.0944)
55°	3.0487	2.6253 E-02	-	-3.6400	-
50°	3.0959	1.5695 E-02	-	-4.1544	-
40°	3.1948	5.0102 E-03	-	-5.2962	-
Arrhenius equation		ln k = 30.4987 - (11200/T)			
Statistic value		r <sup>2</sup> = .9996	V.R.=5215.1640	d.f.= 1,2	Reject H <sub>0</sub> with P =.0003
Heat of Activation, Ea		22,254.40 cal/mol.			
Degradation rate constant estimate to 25°C		shelf life at 25°C follow BP 1980		shelf life at 25°C follow USP XXI	
k <sub>25</sub> (day <sup>-1</sup> )	interval of k <sub>25</sub> (day <sup>-1</sup> )	t <sub>110-90</sub> (month)	interval of t <sub>110-90</sub> (month)	t <sub>130-90</sub> (month)	interval of t <sub>130-90</sub> (month)
8.3742 E-04	1.0090 E-03- 6.9501 E-04	7.99	6.63 - 9.62	14.64	12.14 - 17.64



Table 30 Arrhenius equation of CPC eye drops BPC 1973 containing 20% PEG<sub>8000</sub>.

Temperature		Degradation rate constant of chloramphenicol			
°C	(1/T) X 10 <sup>3</sup> (kelvin <sup>-1</sup> )	k (day <sup>-1</sup> )	interval of k (day <sup>-1</sup> )	ln k	interval of ln k
60°	3.0030	3.4784 E-02	0.0324-0.0371	-3.3586	-3.4293-(-3.2931)
55°	3.0487	2.1082 E-02	-	-3.8593	
50°	3.0959	1.4180 E-02	-	-4.2559	
40°	3.1948	4.3245 E-03	-	-5.4435	
Arrhenius equation		$\ln k = 29.1014 - (10801.81/T)$			
Statistic value		$r^2 = .9959$	V.R.=488.1241	d.f.= 1,2	Reject H <sub>0</sub> with P =0.0015
Heat of Activation, Ea		21463.20 cal/mol.			
Degradation rate constant estimate to 25°C		shelf life at 25°C follow BP 1980		shelf life at 25°C follow USP XXI	
k <sub>25</sub> (day <sup>-1</sup> )	interval of k <sub>25</sub> (day <sup>-1</sup> )	t <sub>110-90</sub> (month)	interval of t <sub>110-90</sub> (month)	t <sub>130-90</sub>	interval of t <sub>130-90</sub> (month)
7.8778 E-04	4.3773 E-04- 1.4177 E-03	8.49	4.7 - 15.28	15.56	8.65 - 28.00

Table 31 Arrhenius equation of CPC eye drops BPC 1973 containing 25% PEG<sub>8000</sub>.

Temperature		Degradation rate constant of chloramphenicol			
°C	(1/T) X 10 <sup>3</sup> (kelvin <sup>-1</sup> )	k (day <sup>-1</sup> )	interval of k (day <sup>-1</sup> )	ln k	interval of ln k
60°	3.0030	3.3615 E-02	0.0312-0.0359	-3.3928	-3.4673-(-3.3270)
55°	3.0487	2.1941 E-02	0.0193-0.0245	-3.8194	-3.9476-(-3.7090)
50°	3.0959	1.3050 E-02	-	-4.3390	-
40°	3.1948	3.6975 E-03	-	-5.6001	-
Arrhenius equation		$\ln k = 31.5771 - (11623.12/T)$			
Statistic value		$r^2 = 0.9955$	V.R.=438.9886	d.f. = 1,2	Reject H <sub>0</sub> with P=0.0016
Heat of Activation, Ea		23095.12 cal/mol.			
Degradation rate constant estimate to 25°C		shelf life at 25°C follow BP 1980		shelf life at 25°C follow USP XXI	
k <sub>25</sub> (day <sup>-1</sup> )	interval of k <sub>25</sub> (day <sup>-1</sup> )	t <sub>110-90</sub> (month)	interval of t <sub>110-90</sub> (month)	t <sub>130-90</sub> (month)	interval of t <sub>130-90</sub>
5.9517 E-04	3.0558 E-04- 1.1593 E-03	11.24	5.77 - 21.89	20.59	10.57 - 40.11

Table 32 Arrhenius equation of CPC eye drops BPC 1973 containing 25% PEG<sub>20000</sub>.

Temperature		Degradation rate constant of chloramphenicol			
°C	(1/T) X 10 <sup>3</sup> (kelvin <sup>-1</sup> )	k (day <sup>-1</sup> )	interval of k (day <sup>-1</sup> )	ln k	interval of ln k
60°	3.0030	2.9756 E-02	0.0273-0.0322	-3.5147	-3.6009-(-3.4358)
55°	3.0487	1.8933 E-02	-	-3.9668	-
50°	3.0959	1.2769 E-02	-	-4.3607	-
40°	3.1948	3.6872 E-03	-	-5.6029	-
Arrhenius equation		ln k = 29.2172 -(10882.13/T)			
Statistic value		r <sup>2</sup> =0.9924	V.R.=260.5709	d.f. = 1,2	Reject H <sub>0</sub> with P =0.0026
Heat of Activation, Ea		21622.79 cal/mol.			
Degradation rate constant estimate to 25°C		shelf life at 25°C follow BP 1980		shelf life at 25°C follow USP XXI	
k <sub>25</sub> (day <sup>-1</sup> )	interval of k <sub>25</sub> (day <sup>-1</sup> )	t <sub>110-90</sub> (month)	interval of t <sub>110-90</sub> (month)	t <sub>130-90</sub> (month)	interval of t <sub>130-90</sub> (month)
6.7552 E-04	3.0046 E-04- 1.5189 E-03	9.90	4.40 - 22.27	18.14	8.07 - 40.79



Table 33 Arrhenius equation of CPC eye drops BPC 1973 containing 30% PEG<sub>20000</sub>.

Temperature		Degradation rate constant of chloramphenicol			
°C	(1/T) X 10 <sup>3</sup> (kelvin <sup>-1</sup> )	k (day <sup>-1</sup> )	interval of k (day <sup>-1</sup> )	ln k	interval of ln k
60°	3.0030	2.9598 E-02	0.0272-0.0319	-3.5200	-3.6045-(-3.4451)
55°	3.0487	1.7791 E-02	0.0152-0.0204	4.0291	-4.1858-(-3.8941)
50°	3.0959	9.5717 E-02	0.0070-0.01214	-4.6489	-4.9618-(-4.4112)
40°	3.1948	3.9889 E-03	-	-5.5242	-
Arrhenius equation		$\ln k = 27.871 - (10468.97/T)$			
Statistic value		$r^2 = 0.9924$	V.R.=261.5758	d.f. = 1,2	Reject H <sub>0</sub> with P = ?
Heat of Activation, Ea		20801.84 cal/mol.			
Degradation rate constant estimate to 25°C		shelf life at 25°C follow BP 1980		shelf life at 25°C follow USP XXI	
k <sub>25</sub> (day <sup>-1</sup> )	interval of k <sub>25</sub> (day <sup>-1</sup> )	t <sub>110-90</sub> (month)	interval of t <sub>110-90</sub> (month)	t <sub>130-90</sub>	interval of t <sub>130-90</sub> (month)
7.0473 E-04	3.1158 E-40 1.9843 E-30	9.49	4.36 - 20.67	17.39	7.99 - 37.88

Table 34 Arrhenius equation of CPC eye drops BPC 1973 containing 15% PF<sub>407</sub>.

Temperature		Degradation rate constant of chloramphenicol			
°C	(1/T) X 10 <sup>3</sup> (kelvin <sup>-1</sup> )	k (day <sup>-1</sup> )	interval of k (day <sup>-1</sup> )	ln k	interval of ln k
60°	3.0030	2.6221 E-02	0.0238-0.0285	-3.6412	-3.7380-(-3.5578)
55°	3.0487	1.7530 E-02	0.0149 -0.0201	-4.0438	-4.2063-(-3.9070)
50°	3.0959	9.3723 E-03	-	-4.6700	-
40°	3.1948	3.3150 E-03	-	-5.7093	-
Arrhenius equation		$\ln k = 29.2944 - (10957.31/T)$			
Statistic value		r <sup>2</sup> =0.9970	V.R.=666.8115	d.f. = 1,2	Reject H <sub>0</sub> with P =0.0011
Heat of Activation, Ea		21,772.18 cal/mol.			
Degradation rate constant estimate to 25°C		shelf life at 25°C follow BP 1980		shelf life at 25°C follow USP XXI	
k <sub>25</sub> (day <sup>-1</sup> )	interval of k <sub>25</sub> (day <sup>-1</sup> )	t <sub>110-90</sub> (month)	interval of t <sub>110-90</sub> (month)	t <sub>130-90</sub> (month)	interval of t <sub>130-90</sub> (month)
5.6703 E-04	3.4053 E-04- 9.4417 E-04	11.80	7.09 - 19.65	21.62	12.98 - 35.99

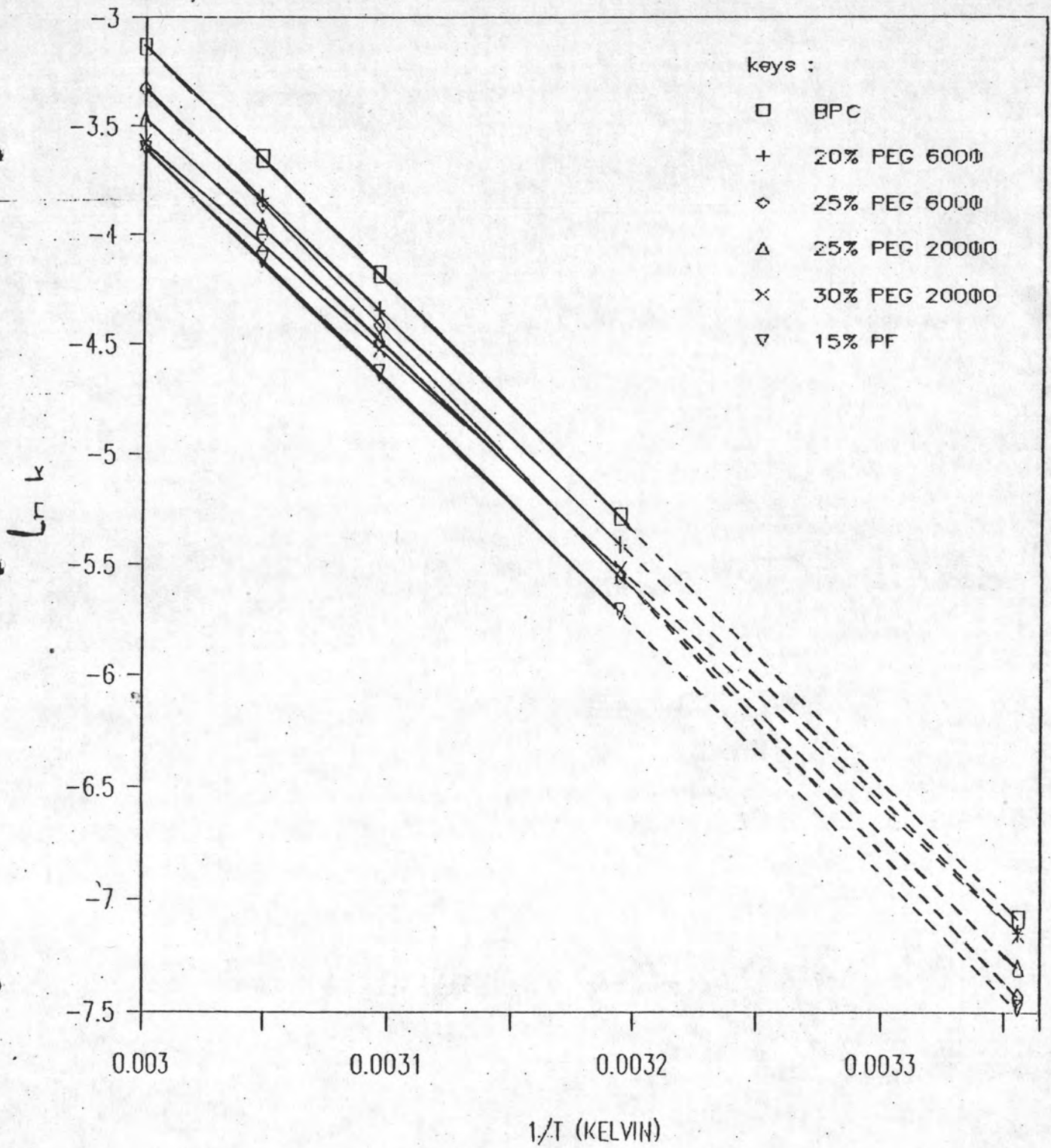


Figure 24 Comparison of calculated Arrhenius plot of the six formulations. The dotted lines were extrapolated to 25°C.



Table 35 Comparison of Heat of Activation ( $E_a$ ) of CPC eye drops.

Formulation	Heat of Activation ( $E_a$ ) (cal/mol.)
BPC	22,254.40
20% PEG <sub>6000</sub>	21,463.20
25% PEG <sub>6000</sub>	23,095.12
25% PEG <sub>20000</sub>	21,622.79
30% PEG <sub>20000</sub>	20,801.84
15% Poloxamer <sub>407</sub>	21,772.18



Table 36 Comparison of the degradation rate constant extrapolated to 25°C, and shelf-life calculated according to the standard of BP 1980 and USP XXI.

Formulation	Degradation rate constant extrapolated to 25°C		shelf life at 25°C according to BP 1980 (110-90% LA)		shelf life at 25°C according to USP XXI (130-90% LA)	
	$k_{25} \times 10^{-4}$ (day <sup>-1</sup> )	interval of $k_{25} \times 10^{-4}$ (day <sup>-1</sup> )	$t_{110-90}$ (month)	interval of $t_{110-90}$	$t_{130-90}$ (month)	interval of $t_{130-90}$
BPC	8.3742	6.9501 - 10.0899	7.99	6.63 - 9.62	14.64	12.14 - 17.64
20% PEG <sub>8000</sub>	7.8778	4.3773 - 14.1772	8.49	4.7 - 15.28	15.56	8.65 - 28.00
25% PEG <sub>8000</sub>	5.9517	3.0558 - 11.5934	11.24	5.77 - 21.89	20.59	10.57 - 40.11
25% PEG <sub>20000</sub>	6.7552	3.0046 - 15.1885	9.90	4.40 - 22.27	18.14	8.07 - 40.79
30% PEG <sub>20000</sub>	7.0473	3.1158 - 19.8467	9.49	4.36 - 20.67	17.39	7.99 - 37.88
15% PF <sub>407</sub>	5.6703	3.4053 - 9.4417	11.80	7.09 - 19.65	21.62	12.98 - 35.99

Table 37 Comparison of the degradation rate constant extrapolated to 8°C , and shelf - life calculated according to the standard of BP 1980 and USP XXI.

Formulation	Degradation rate constant extrapolated to 8°C		shelf life at 8°C according to BP 1980 (110-90% LA)		shelf life at 8°C according to USP XXI (130-90% LA)	
	$k_d \times 10^{-5}$ (day <sup>-1</sup> )	interval of $k_d$ (day <sup>-1</sup> )	$t_{110-90}$ (month)	interval of $t_{110-90}$	$t_{130-90}$ (month)	interval of $t_{130-90}$
BPC	8.6195	6.2647 - 11.8642	77.61	56.39 - 106.79	142.20	103.13 - 195.65
20% PEG <sub>6000</sub>	8.7909	3.2124 - 24.0569	76.10	27.81 - 208.25	139.42	50.95 - 381.54
25% PEG <sub>6000</sub>	5.6217	1.7939 - 17.6168	119.00	37.98 - 372.92	218.03	69.58 - 683.22
25% PEG <sub>20000</sub>	7.4166	1.8509 - 29.7192	90.20	22.51 - 361.45	165.26	41.24 - 662.21
30% PEG <sub>20000</sub>	8.4139	2.2180 - 31.9211	79.51	20.96 - 301.62	145.67	38.40 - 552.59
15% PF <sub>407</sub>	6.1309	2.5590 - 14.6879	109.12	45.55 - 261.43	199.92	83.45 - 478.96



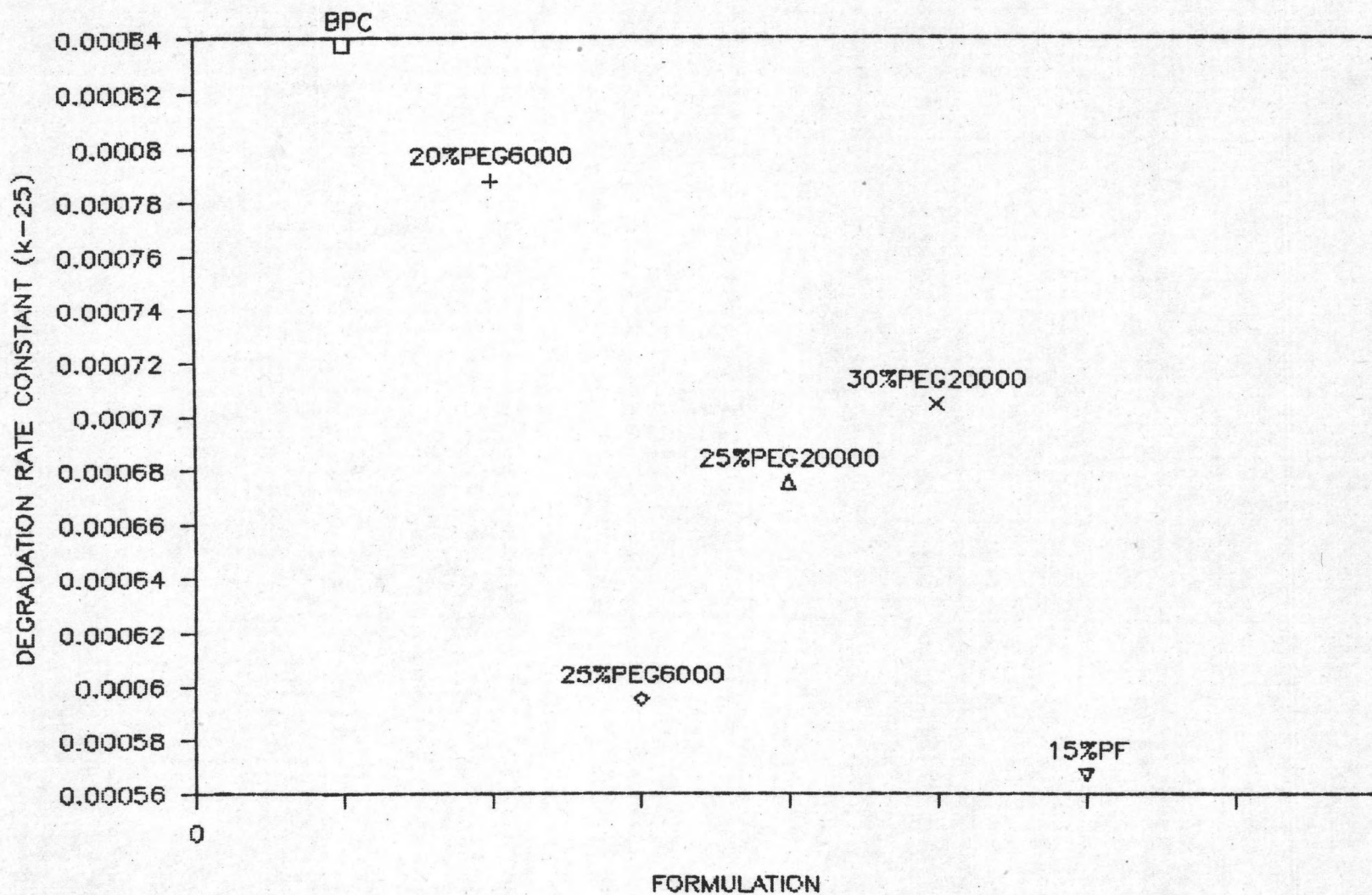


Figure 25 Comparison of rate constants of CPC eye drops at 25°C.

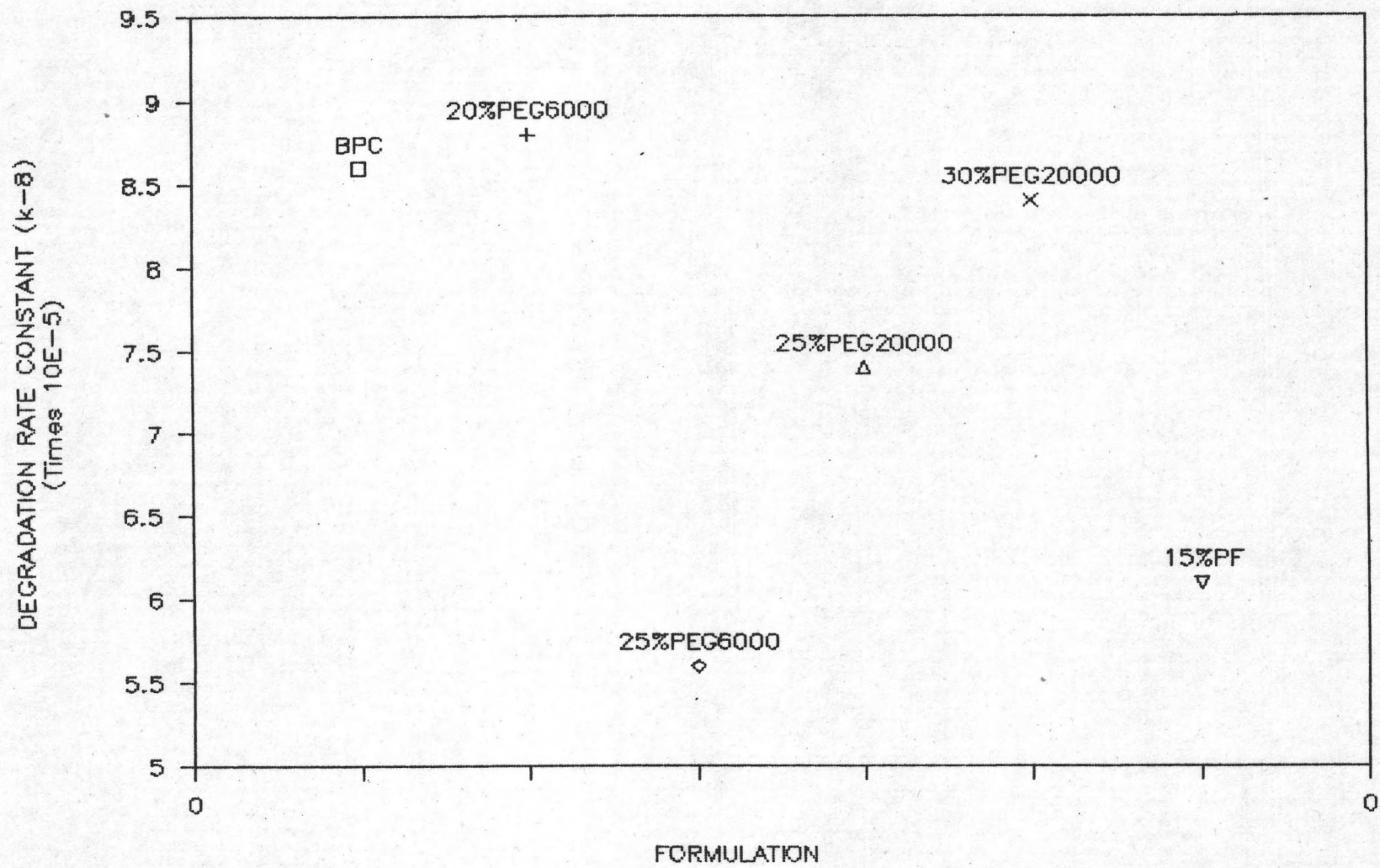


Figure 26 Comparison of rate constants of CPC eye drops at 8°C.

The shelf-life of the six formulations calculated according to standard of BP 1980 and USP XXI were compared in Tables 36-37 and Figures 27-28. At both 25° and 8°C the rank order of shelf-life was opposite to the order of rate constant.



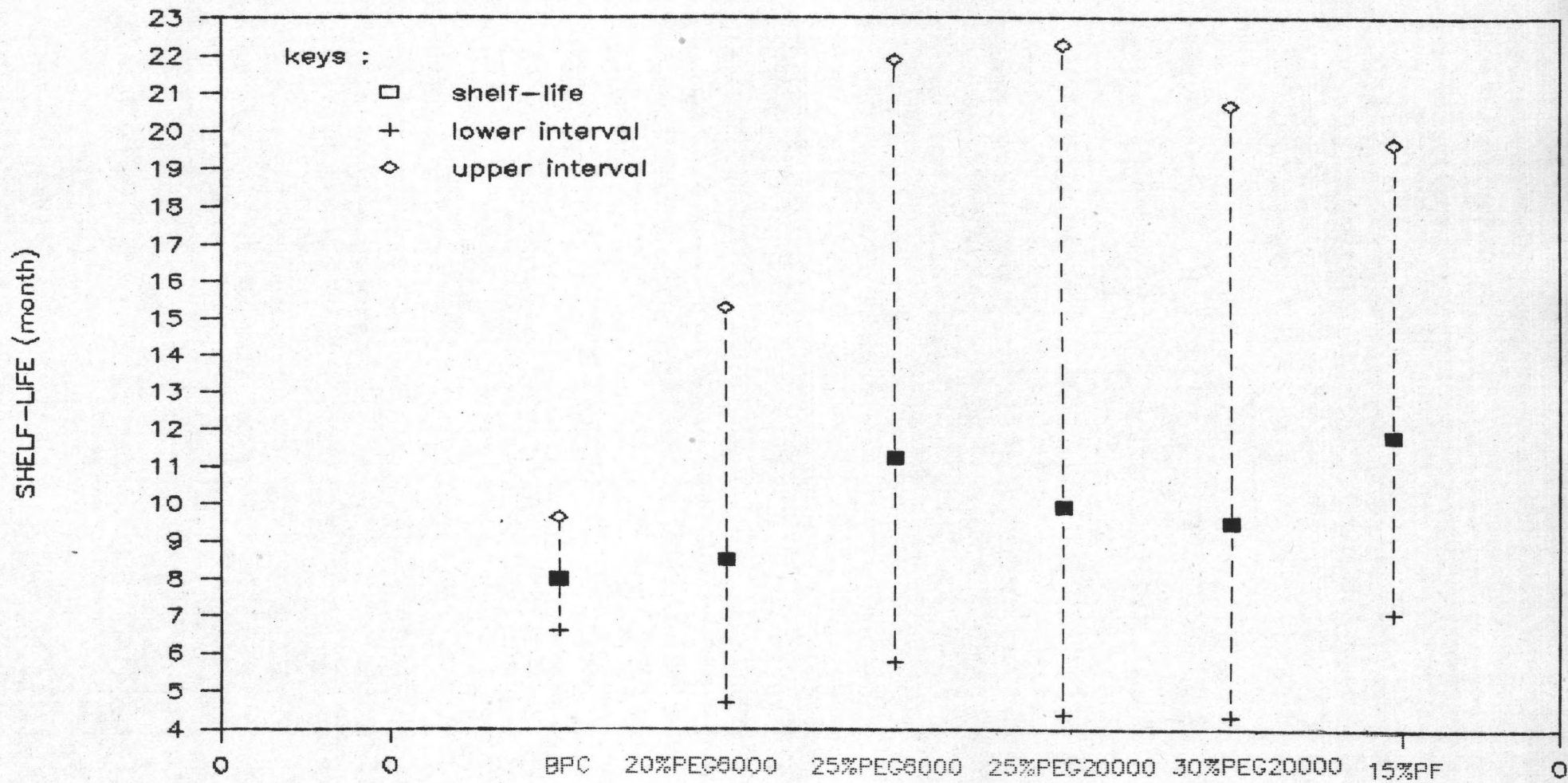


Figure 27 Comparison of shelf-life of CPC eye drops at 25°C calculated according to the standard of BP 1980 ( $t_{110-90}$ ).

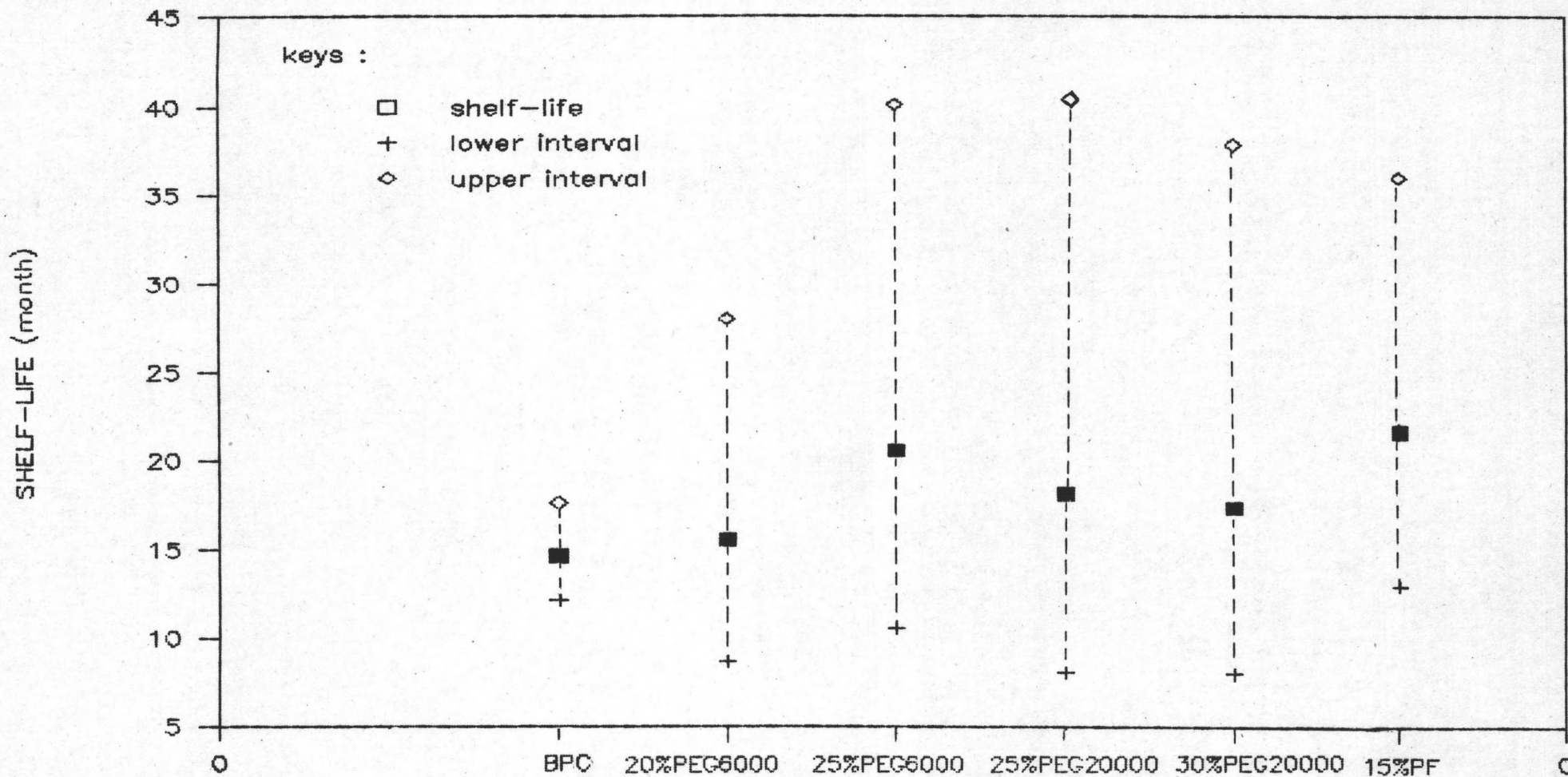


Figure 28 Comparison of shelf-life of CPC eye drops at 25°C calculated according to the standard of USP XXI ( $t_{130-90}$ ).