CHAPTER IV

RESULTS AND DISCUSSION

Analyses of Nifedipine

Majeed et al. (1987) and Al-Turk et al. (1989) reported that the spectra of nifedipine in 95% ethanol showed absorption maxima at 237 and 360 nm before irradiation under fluorescent light and the absorption spectrum of the same solution after irradiation showed a decrease in the absorption maxima at 237 and 360 nm and the new maximum at 280 nm. In other study (Al-Turk et al., 1988), in addition to 280 nm, the spectrum of oxidized form (after irradiation) was also found at 310 nm.

In this investigation, photo-oxidation of nifedipine under fluorescent light was accomplished. The absorption spectrum of 3.403×10^{-5} M of nifedipine in 95% ethanol containing 40% w/w Pluronic F-127 gel (Appendix B) showed absorption maximum at 334 nm before irradiation. This pre-irradiated solution and its corresponding spectrum were referred to as the reduced form, which was reported to be the dihydropyridine (Al-Turk et al., 1989). The absorption spectrum after 4 hours of irradiation showed a decrease in the absorption maximum at 334 nm and the appearance of the new

absorption maxima at 281 and 310 nm (Appendix B). The post-irradiated product and its corresponding spectrum were referred to as the oxidized form, which was reported to be corresponding to the nitrosopyridine product (Al-Turk et al., 1989).

In the presence of sodium bisulfite, the absorption spectra before and after irradiation were similar to which of nifedipine solution in the absence of sodium bisulfite. At all concentrations of sodium bisulfite, the spectra before and after irradiation were found in the same pattern. However, Al-Turk et al. (1988) found that there was a change in the spectrum after irradiation of nifedipine when sodium bisulfite was added to the solution of nifedipine. They found that after irradiation, there was a decrease in the absorption maxima at 237 and 360 nm and new absorption maximum at 274 nm appeared, there was no absorption peak at 310 nm which was found in the spectrum of nifedipine solution without sodium bisulfite.

The analysis of nifedipine in this investigation was based on the measurement of absorbance values at two wavelengths which included 334 and 281 nm. The concentration of nifedipine in the preparation was then calculated by solving for the equation which was specific for individual preparation (Appendix B).

Photostability Study of Nifedipine Gels

A. Physical Stability Study

Physical appearances, including color and pH, of seven nifedipine gel preparations before exposure to light were shown in Table 1. The 40% w/w Pluronic F-127 gel was transparent and colorless, and after addition of nifedipine, the 40% w/w Pluronic F-127 gel turned yellow. All formulations containing sodium bisulfite were still yellow. Initially, the pH of nifedipine gel was 6.96, and the values decreased in the formulations containing sodium bisulfite at various concentrations. The higher concentration of sodium bisulfite was added, the lower pH was. It might be due to the acidic property of sodium bisulfite (Akers, 1982).

Visual observation of color change of all nifedipine gel formulations after exposure to light were presented in Table 2. Nifedipine gel (formulation Ia) which exposed to accelerated light acted as a control. On exposure to normal and accelerated light, the yellow color of nifedipine gels was found to change to dark yellow and brownish yellow, respectively. Comparing with accelerated light, normal light produced less dark yellow of nifedipine gel, while on exposure to accelerated light, the color of nifedipine gel changed until brownish

Table 1 Physical Appearances of Seven Nifedipine Gels Before Exposure to Light

Formulations[a]	Color	pH
Ia	yellow	6.96
Ib	yellow	6.96
Ic	yellow	6.96
II	yellow	6.52
III	yellow	6.35
IV	yellow	5.90
V	yellow	5.55

[a] Ia = Nifedipine gel, under accelerated light

Ib = Nifedipine gel, under normal light

Ic = Nifedipine gel wrapped in aluminium foil, under accelerated light

II = Nifedipine gel containing sodium bisulfite 0.05% w/w, under accelerated light

III = Nifedipine gel containing sodium bisulfite 0.10% w/w, under accelerated light

IV = Nifedipine gel containing sodium bisulfite 0.30% w/w, under accelerated light

V = Nifedipine gel containing sodium bisulfite 0.50% w/w, under accelerated light

Table 2 Color Change of Seven Nifedipine Gels After Exposure to Light for 1, 34, 56, 86 and 116 Days.

Formulations \ Time (Days)	Color[a]				
	1	34	56	86	116
Ia	+4	+6	+6	+6	+6
Ib	+3	+3	+3	+3	+3
Ic	0	0	0	0	0
II	-1	-1	-1	+2	+2
III	-1	-1	-1	+1	+1
IV	-1	-1	-1	+1	+1
V	+5	+7	+7	+7	+7

[a] (-) : The number of (-) showed a degree of pale yellow

0 : yellow

(+) : The number of (+) showed a degree of dark yellow, e.g., +1 = dark yellow, +6 = brownish yellow

yellow appeared. The darkening of both formulations could probably be ascribed to oxidation of nifedipine (Al-Turk et al., 1988).

On exposure to accelerated light, formulations II-V with sodium bisulfite 0.05, 0.10, 0.30 and 0.50 % w/w appeared to change in color. While the formulations containing 0.05, 0.10 and 0.30 % w/w of sodium bisulfite changed to pale yellow, that with 0.50% w/w changed to dark yellow on the first day of exposure and then to brownish yellow later, which seemed to be deeper than the color change in the control. The pale yellow of nifedipine gels containing sodium bisulfite 0.05, 0.10 and 0.30 % w/w (formulations II-IV) appeared for more than 2 months and then changed to dark yellow which was less than that of the control.

On the other hand, yellow color of nifedipine gel wrapped in aluminium foil (formulation Ic) was unchanged through 116 days of exposure to accelerated light. From the results, it might be said that only formulation Ic, according to physical stability study, was stable to photo-oxidation of nifedipine.

Many factors affected stability of dyes (Sprowls, ed., 1970), such as light, alkali, acid, oxidizing agents, reducing agents (Schroeter, 1961) and

chemical constitution (Lachman et al., 1980). In this study, the difference of color of nifedipine gels, which appeared after exposure to light in such formulations containing sodium bisulfite, might be due to the different pH of formulations which resulted from the different concentration of sodium bisulfite.

B. Chemical Stability Study

Amounts of nifedipine before exposure to light and % labeled amounts of all formulations of nifedipine gel were shown in Table 3. Table 4 showed amounts of nifedipine in all various formulations after exposure to light, presented as % remaining of nifedipine as a function of time. In this study, nifedipine gel which exposed to accelerated light (formulation Ia) was compared with other formulations as control. From Table 4, decrement of nifedipine concentrations appeared in all formulations except nifedipine gel wrapped in aluminium foil (formulation Ic).

To determine the reaction kinetics and the order of reaction, plots of % remaining of nifedipine versus time, ln of % remaining of nifedipine versus time and $(\%\ \text{remaining})^{-1}$ versus time were performed. Linear regression was used to determine the coefficients of determination (r^2) which were then compared as shown in

Table 3 Amounts of Nifedipine and % Labeled Amounts (% LA) of Seven Nifedipine Gels

Formulations	Amount of Nifedipine[a] (mg/g)	% LA[a]
Ia	10.19 ± 0.09	101.93 ± 0.97
Ib	10.99 ± 0.09	109.87 ± 0.86
Ic	11.71 ± 0.08	117.13 ± 0.80
II	10.35 ± 0.15	103.50 ± 1.47
III	10.33 ± 0.07	103.30 ± 0.70
IV	10.65 ± 0.07	106.53 ± 0.76
V	10.66 ± 0.07	106.57 ± 0.66

a = Mean ± S.D. (n = 3)

Table 4 Percent Remaining of Nifedipine in Seven Nifedipine Gels as a Function of Time

Formulations / Time (Days)	Percent Remaining[a]			
	Ia	Ib	Ic	II
0	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
0.02	97.16±1.00	-	102.22±1.49	95.76±1.34
0.04	92.71±0.10	-	99.98±1.93	91.02±0.99
0.08	86.26±4.83	-	101.57±0.92	82.07±1.01
0.17	69.79±1.67	-	100.83±0.86	67.47±0.26
0.33	41.42±2.74	-	101.48±6.04	48.06±0.97
1	10.55±1.40	81.80±2.19	101.14±1.50	11.53±0.16
1.25	5.76±0.86	-	100.20±1.09	9.32±0.74
2	4.22±0.41	62.38±1.33	99.46±1.04	9.80±0.86
3	4.35±0.34	48.37±2.33	100.29±1.08	9.88±0.64
4	3.99±0.18	39.69±1.30	99.75±1.56	10.40±0.38
8	5.63±0.42	14.39±0.98	99.80±0.79	10.25±0.68
12	5.56±0.36	6.80±0.34	100.68±2.68	9.53±1.10
16	4.45±0.11	6.40±0.31	100.32±2.26	9.55±0.76
20	5.32±0.52	7.04±0.50	99.04±2.32	10.05±0.98
27	5.26±0.62	6.40±0.87	100.21±2.27	9.98±0.85
34	5.00±0.16	5.89±1.02	99.30±2.56	9.99±0.43
41	5.23±0.28	5.52±0.51	100.57±1.34	9.43±0.50
56	4.87±0.65	6.58±0.14	100.37±1.21	10.02±1.02
71	4.61±0.59	6.53±0.38	100.31±0.42	9.83±1.17
86	5.17±0.26	6.59±0.52	100.11±0.73	10.53±0.46
116	5.39±0.23	6.19±0.86	100.52±1.15	10.44±0.48

a = Mean ± S.D. (n = 3)

Table 4 (continued)

Formulations / Time (Days)	Percent Remaining[a]		
	III	IV	V
0	100.00±0.00	100.00±0.00	100.00±0.00
0.02	97.51±1.34	97.15±0.34	97.69±0.38
0.04	94.64±0.14	96.34±0.31	96.09±0.62
0.08	87.74±0.89	93.02±0.78	92.74±0.11
0.17	77.99±0.14	85.17±1.05	85.05±0.29
0.33	62.60±0.73	73.10±0.94	73.67±0.52
1	26.04±0.86	41.14±0.74	40.32±0.45
1.25	20.72±0.42	32.79±0.63	32.10±0.41
2	20.98±0.92	31.50±2.07	32.47±0.53
3	20.75±0.48	31.23±1.50	32.28±0.93
4	22.75±1.47	31.57±0.36	30.62±0.20
8	22.10±0.82	30.32±0.77	31.75±0.80
12	21.71±1.53	29.23±0.45	32.03±0.73
16	20.26±0.50	31.13±1.25	30.78±2.38
20	22.14±1.20	30.56±1.64	28.40±0.51
27	23.33±0.88	30.82±1.91	28.75±0.49
34	22.63±2.05	30.81±1.98	28.78±1.06
41	22.39±1.64	29.10±0.69	29.09±0.34
56	21.20±0.91	28.51±0.74	28.47±0.78
71	23.17±0.34	29.29±0.31	28.74±0.46
86	20.39±0.70	31.51±1.37	29.31±0.70
116	21.39±0.74	31.39±0.83	29.96±1.86

a = Mean ± S.D. (n = 3)

Table 5. The results indicated that the coefficients of determination which were nearest to 1 were obtained from plot of ln of % remaining versus time. Therefore, it would seem that the photodegradation followed first-order reaction. The degradation of nifedipine gel was exponential as the following equation :-

$$\ln C = \ln C_o - kt$$

Similar results were obtained by Jakobsen, Pedersen and Mikkelsen (1979), and, Thoma and Klimek (1985 b). In their studies on photodegradation of nifedipine solution, the reaction followed first-order kinetics. Another study (Tucker, Minty and MacGregor, 1985) also showed that nifedipine in whole blood, plasma and distilled water, the reaction followed first-order degradation kinetics and this type of reaction was also presented in photodegradation of film coated nifedipine (Teraoka, Matsuda and Sukimoto, 1988). However, different results, zero-order kinetics were obtained by Akimoto et al. (1988) and Majeed et al. (1987) in the studies on photodegradation of nifedipine solutions.

By plotting ln of % remaining of nifedipine versus time, straight line curves were obtained as shown

Table 5 Coefficients of Determination (r^2) of the Relationship Between % Drug Remaining versus Time, ln % Drug Remaining versus Time, (% Drug Remaining)$^{-1}$ versus Time.

Formulations	Coefficients of Determination (r^2)[a]		
	% Drug Remaining versus Time	ln % Drug Remaining versus Time	(% Drug Remaining)$^{-1}$ versus Time
Ia	0.9099	0.9978	0.9448
Ib	0.8629	0.9971	0.9301
II	0.9219	0.9933	0.9838
III	0.9627	0.9978	0.9926
IV	0.9855	0.9997	0.9875
V	0.9871	0.9999	0.9934

a = The results obtained from 3 samples.

in Figures 1 to 7. The plots for the samples exposed to accelerated light and normal light (formulations Ia, II-V and Ib, respectively) showed two different slopes. On exposure to accelerated light, the curves levelled off between 1 and 2 days and showed no further degradation. On exposure to normal light, the curve levelled off on the 12 th day and showed no further degradation. Similar result was observed by Thoma and Klimek (1985 b) in their study on photoinstability of crystalline nifedipine. From their study, nifedipine degraded to 80% of original in 40 minutes after exposure to light and no further degradation. In this study, the changes in reaction rates that were observed as the duration of irradiation was increased might be due to the equilibrium of nifedipine in gel preparations. Therefore, it was decided to utilize the initial degradation rate as accurately representing the photodegradation of nifedipine gels.

Photodegradation occurred between 0-12 days after exposure to light of various formulations of nifedipine gel were compared with the control (formulation Ia) as shown in Figure 8. It was illustrated that normal light caused a different pattern from accelerated light. Irradiation under normal light was limited at only 10-12 hours per day in daytime and

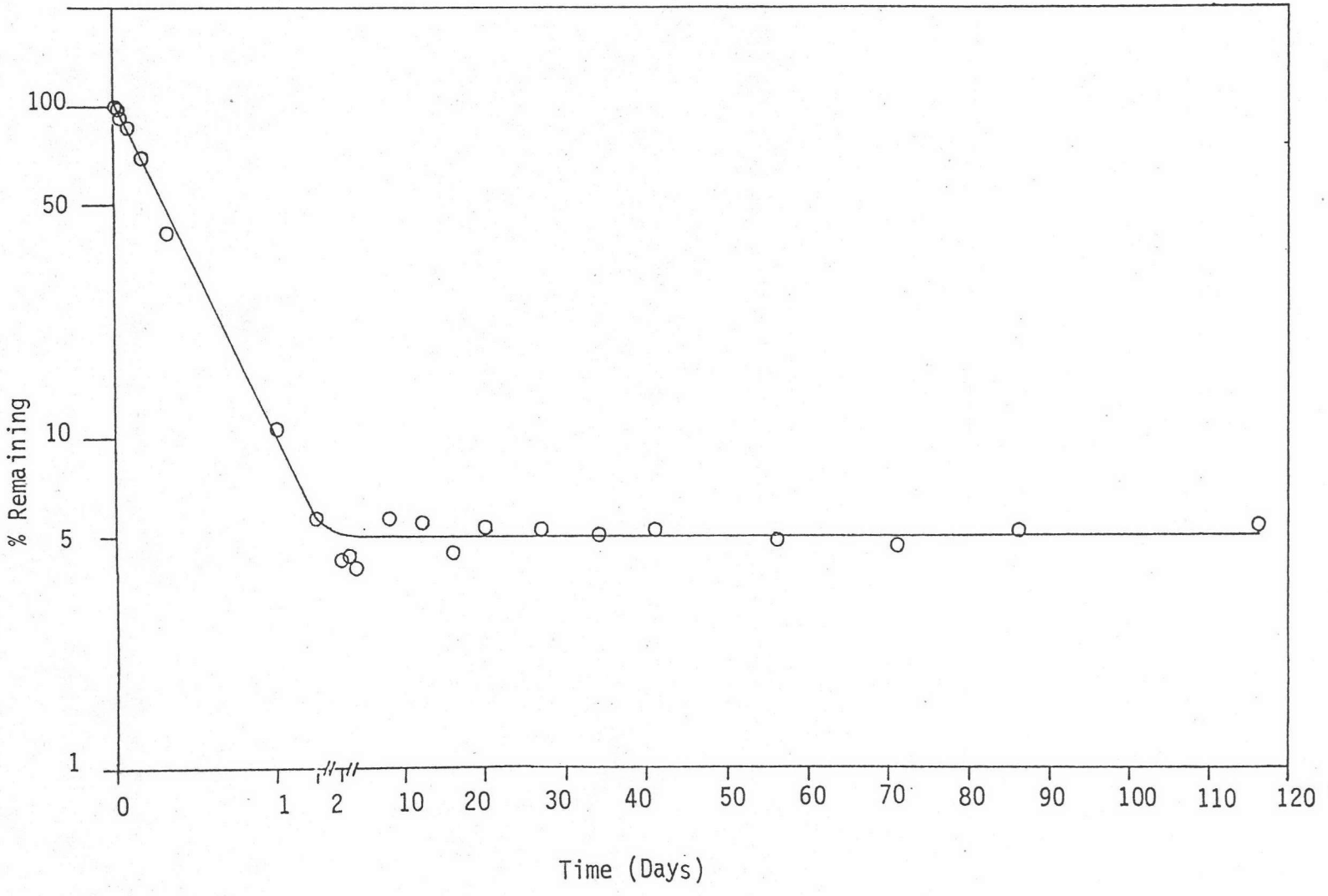

Figure 1 Degradation of Nifedipine in Nifedipine Gel Under Accelerated Light

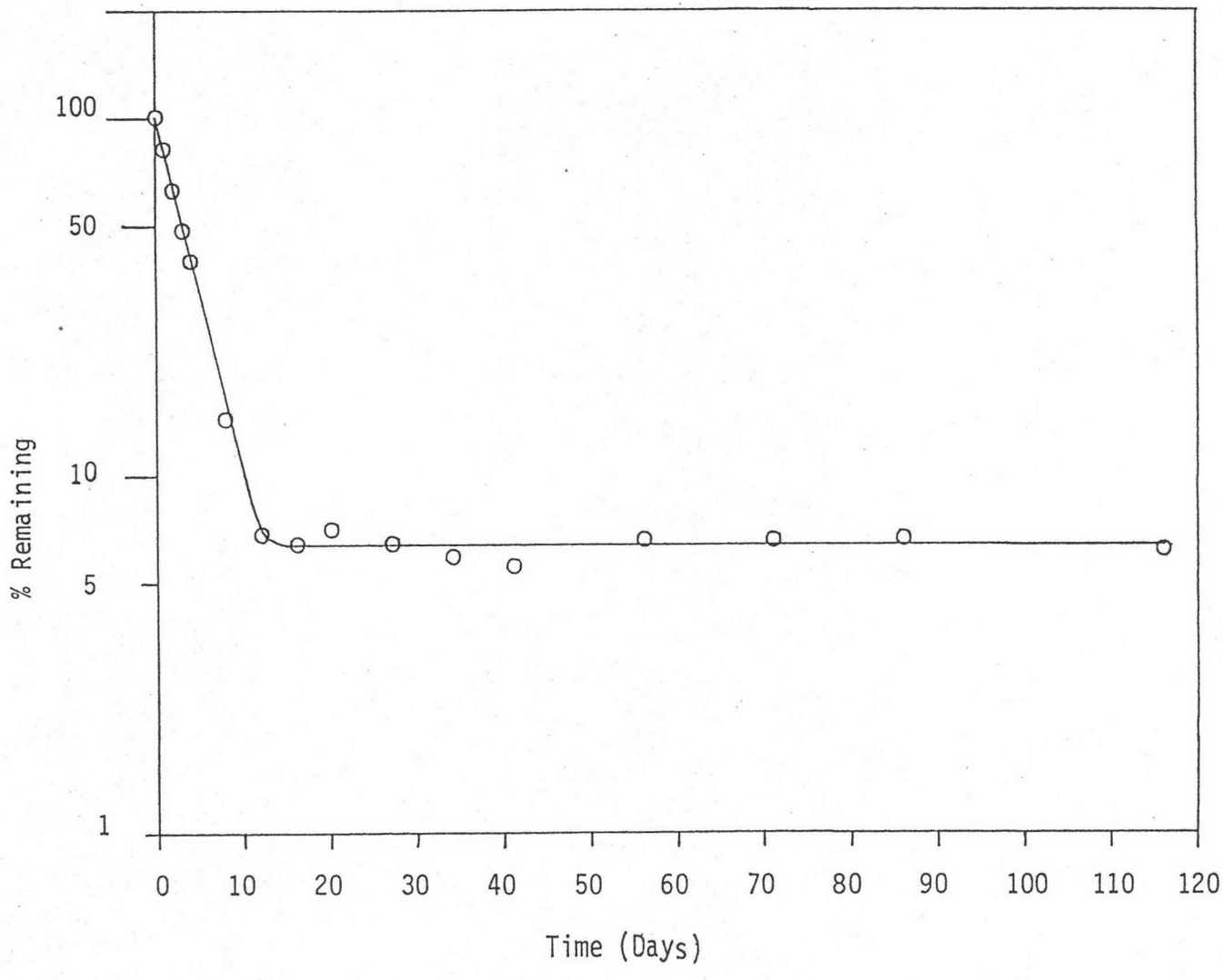

Figure 2 Degradation of Nifedipine in Nifedipine Gel Under Normal Light

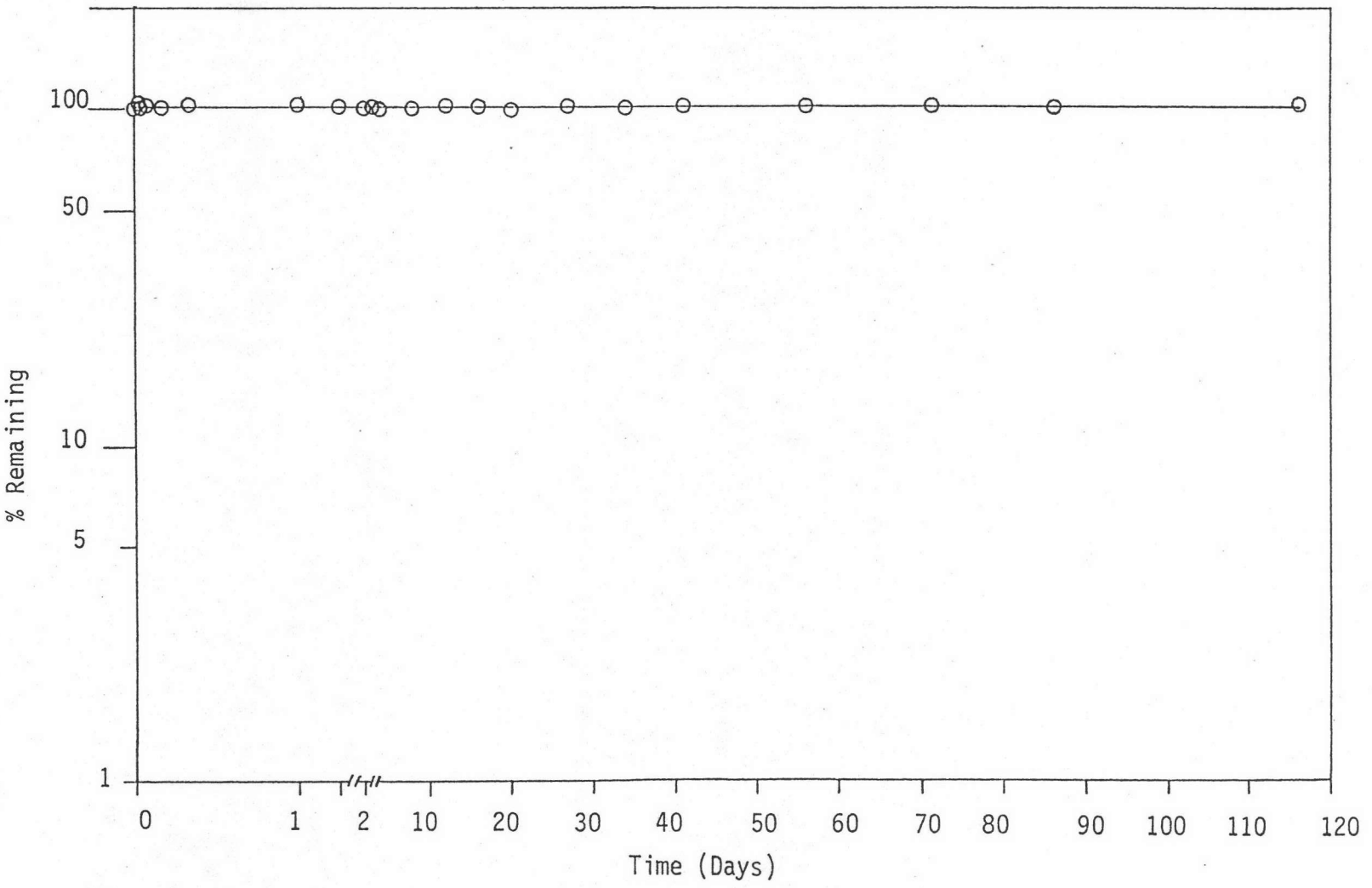

Figure 3 Stability of Nifedipine in Nifedipine Gel Wrapped in Aluminium Foil Under Accelerated Light

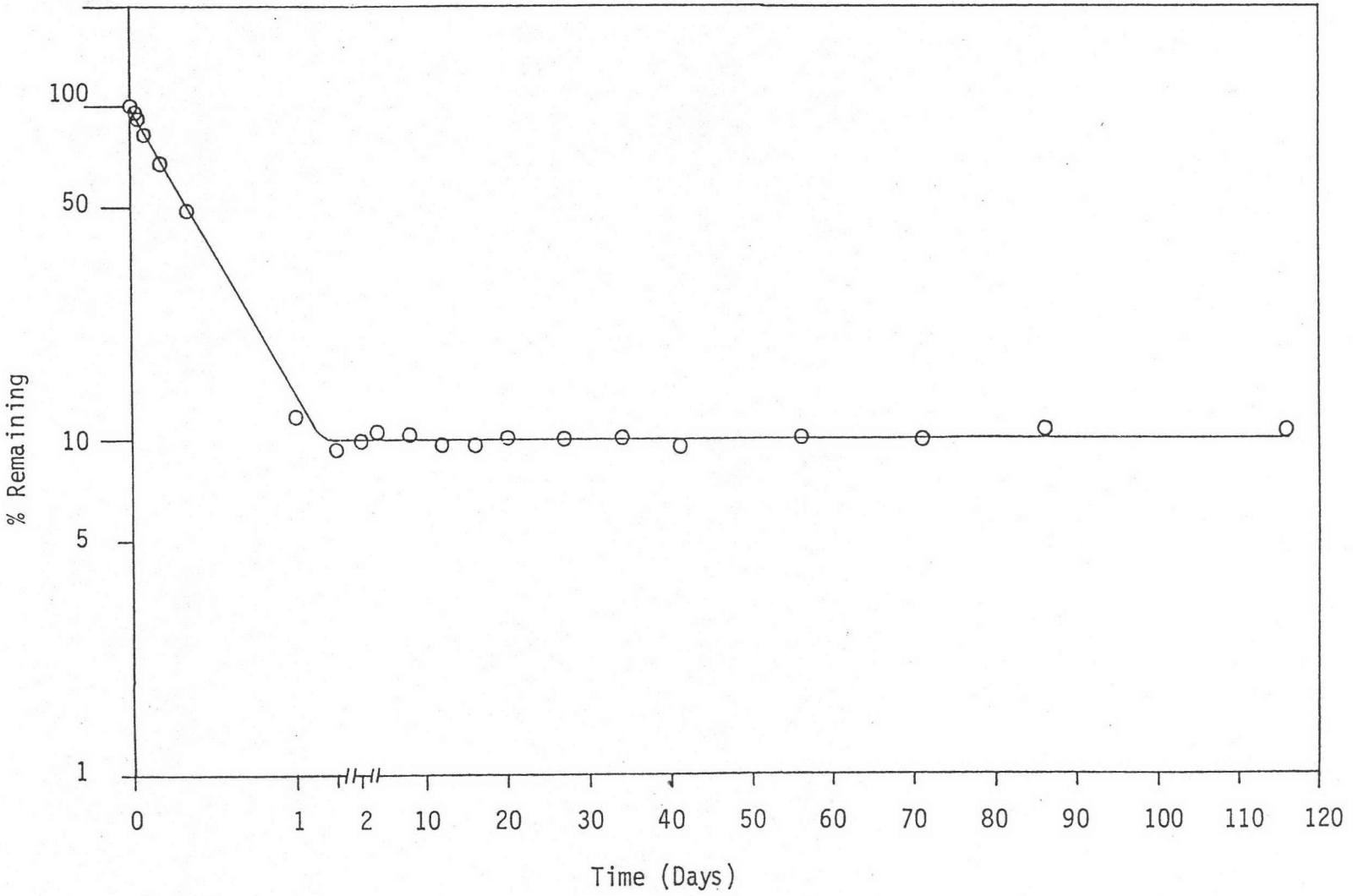

Figure 4 Degradation of Nifedipine in Nifedipine Gel Containing Sodium Bisulfite 0.05% w/w Under Accelerated Light

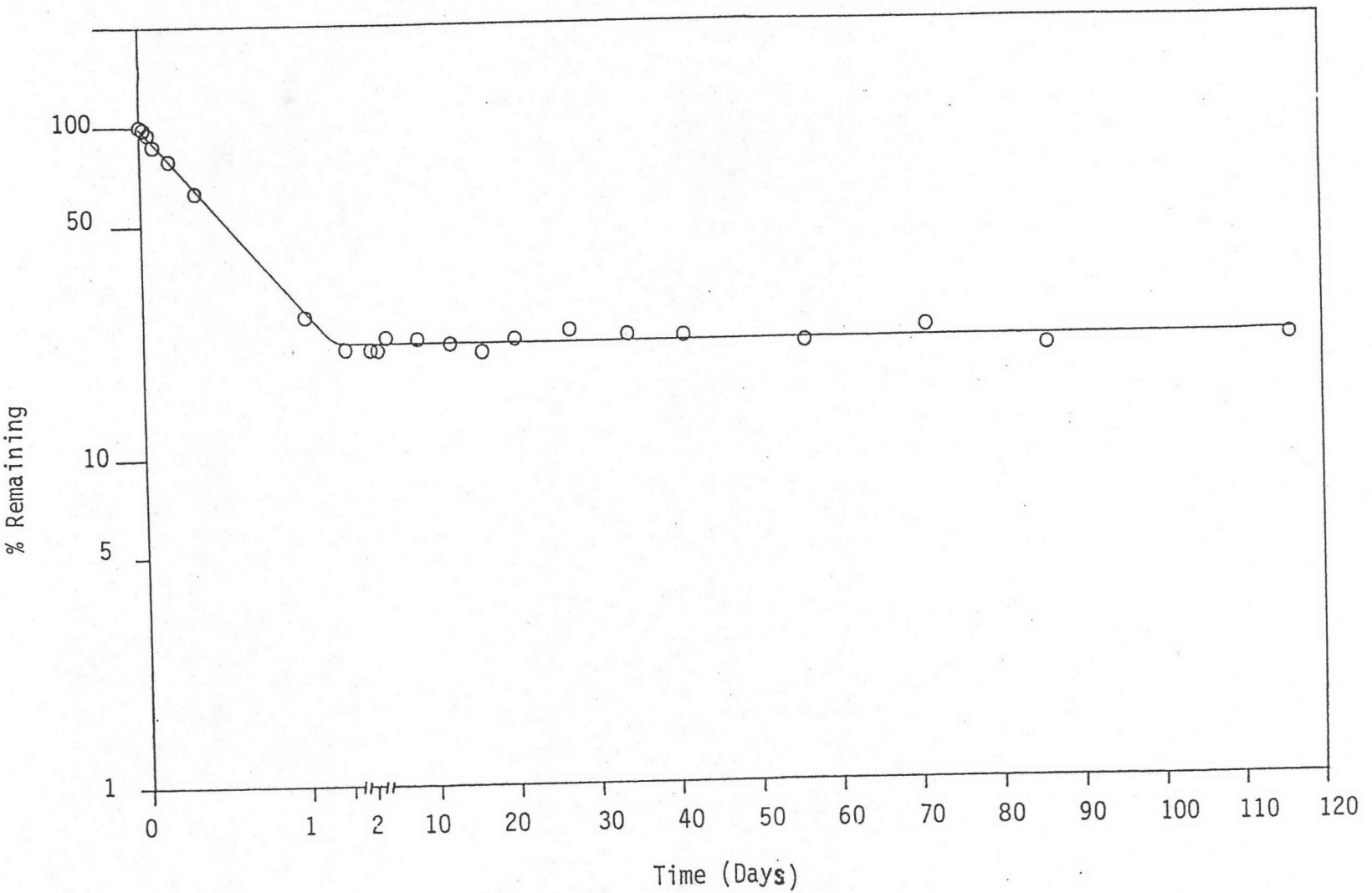

Figure 5 Degradation of Nifedipine in Nifedipine Gel Containing Sodium Bisulfite 0.10% w/w Under Accelerated Light

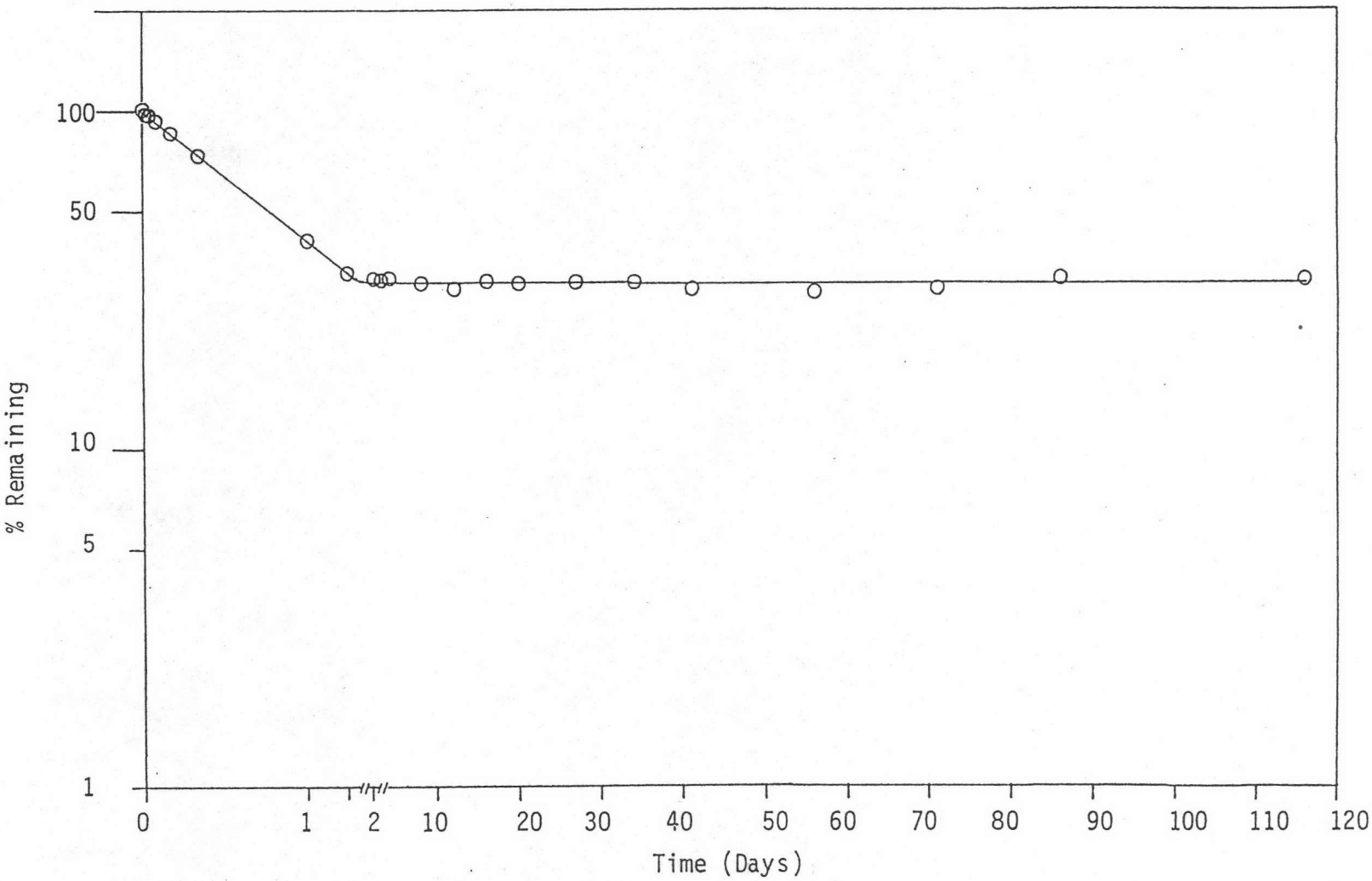

Figure 6 Degradation of Nifedipine in Nifedipine Gel Containing Sodium Bisulfite 0.30 % w/w

Under Accelerated Light

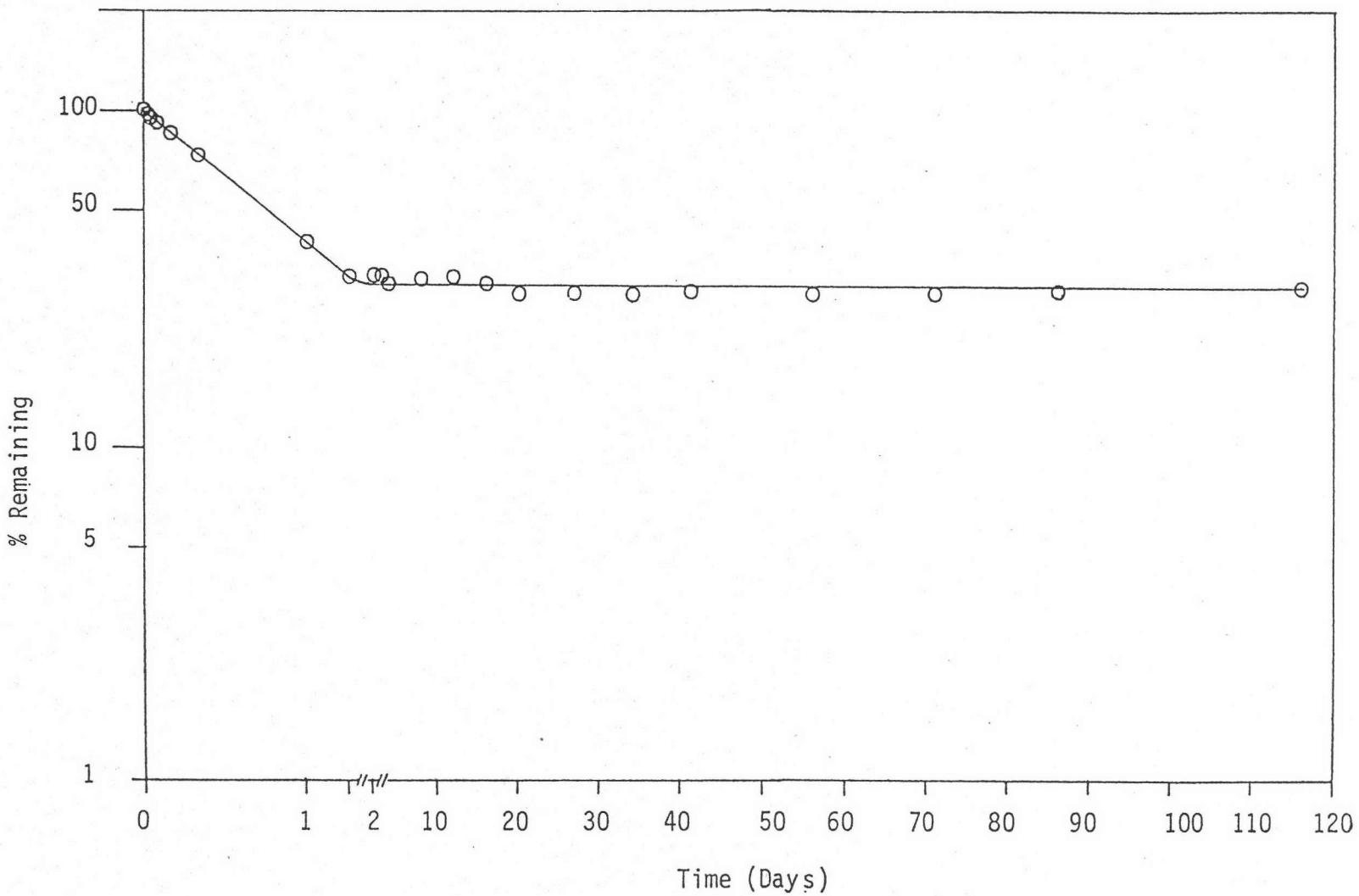

Figure 7 Degradation of Nifedipine in Nifedipine Gel Containing Sodium Bisulfite 0.50% w/w Under Accelerated Light

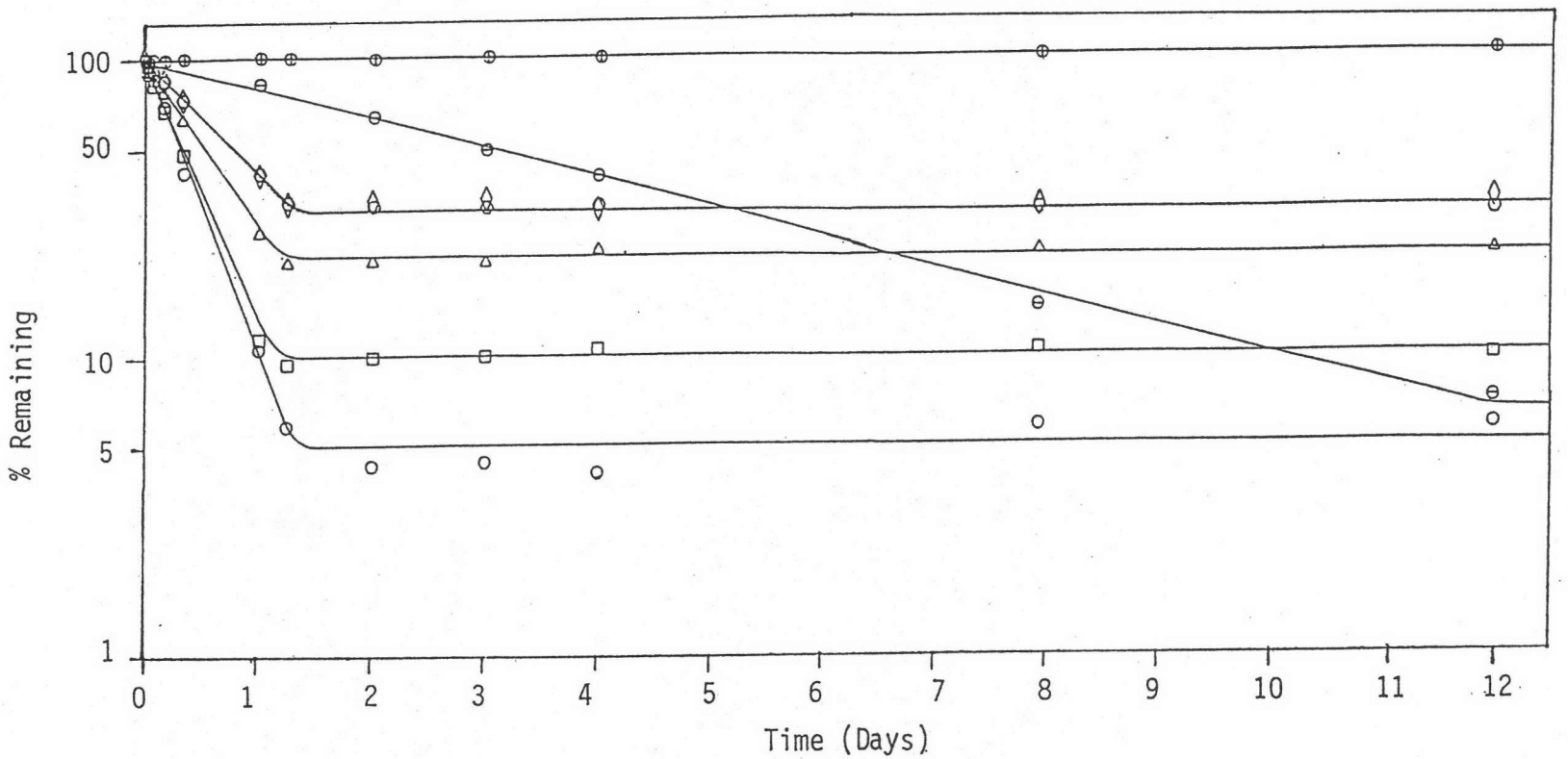

Figure 8 Comparison of the Degradation of seven nifedipine Gels on 0-12 th Day

Keys :

- ○ Nifedipine gel, accelerated light
- ⊖ Nifedipine gel, normal light
- ⓓ Nifedipine gel wrapped in aluminium foil, accelerated light
- □ Nifedipine gel containing sodium bisulfite 0.05% w/w,accelerated light
- △ Nifedipine gel containing sodium bisulfite 0.10% w/w,accelerated light
- ⬡ Nifedipine gel containing sodium bisulfite 0.30% w/w,accelerated light
- ◊ Nifedipine gel containing sodium bisulfite 0.50% w/w,accelerated light

this was the factor that probably contributed to the difference of the pattern of photodegradation caused by normal and accelerated light. Lachman et al. (1960) found that color fading produced by accelerated light was not necessary to be the same pattern of normal light.

Table 6 showed degradation rate constants of all various nifedipine gels. Degradation rate constants of nifedipine gels exposed to normal light (formulation Ib) and wrapped in aluminium foil (formulation Ic) were less than that under accelerated light (formulation Ia) ($P < .05$) as shown in Table 7. The results indicated that aluminium foil could protect nifedipine gel from photo-oxidation. One Way ANOVA and Duncan's New Multiple Range Test for comparison of degradation rate constants of nifedipine gels containing various concentrations of sodium bisulfite were shown in Tables 8 and 9. There were statistically significant differences among formulations Ia, II, III, IV and V ($P < .05$). However, there was no statistically significant difference between formulations IV and V ($P > .05$). In contrast, Al-Turk et al. (1988) found that there was no significant change in the rate of disappearance of nifedipine in solution with respect to sodium bisulfite concentration between 6×10^{-5} M to 500×10^{-5} M.

Table 6 Degradation Rate Constants of Seven Nifedipine Gels

Formulations	Degradation Rate Constants $(Days^{-1})^a$
Ia	2.2895 ± 0.1425
Ib	0.2285 ± 0.0049
Ic	0.0000 ± 0.0000
II	1.9777 ± 0.0458
III	1.2824 ± 0.0223
IV	0.8867 ± 0.0189
V	0.9059 ± 0.0101

a = Mean ± S.D. (n = 3)

Table 7 Comparison of Degradation Rate Constants of Two Formulations (Nifedipine Gel Under Normal Light and Nifedipine Gel Wrapped in Aluminium Foil Under Accelerated Light) with Nifedipine Gel Under Accelerated Light, Using Student's t-test.

Formulations	t-value (calculated) Comparison with Nifedipine Gel Under Accelerated Light	Statistical Significance
Nifedipine Gel Under Normal Light	25.1341	S
Nifedipine Gel Wrapped in Aluminium Foil Under Accelerated Light	27.9207	S

$t^{a}_{(.05,\ 4)} = 2.776$

S = significant at $P < .05$

a = t-value from the table

Table 8 Analysis of Variance for Degradation Rate Constants of Nifedipine Gels with Various Concentrations of Sodium Bisulfite

Source of Variation	df[a]	SS[b]	MS[c]	F[d]
Among Groups	4	4.8687	1.2172	258.9787
Within Group	10	0.0468	0.0047	
Total	14	4.9155		

$$F^{e}_{.05(4,\ 10)} = 3.48$$

a = degree of freedom

b = Sum of Square

c = Mean Square

d = Variance Ratio

e = F-value obtained from the table

Table 9 Comparison of Degradation Rate Constants of Nifedipine Gels with Various Concentrations of Sodium Bisulfite, Using Duncan's New Multiple Range Test.

Formulations	Difference Between Means	LSR[a]	Statistical Significance
Ia versus II	0.3118	0.1247	S
Ia versus III	1.0071	0.1307	S
Ia versus IV	1.4028	0.1358	S
Ia versus V	1.3836	0.1334	S
II versus III	0.6953	0.1247	S
II versus IV	1.0910	0.1334	S
II versus V	1.0718	0.1307	S
III versus IV	0.3957	0.1307	S
III versus V	0.3765	0.1247	S
IV versus V	0.0192	0.1247	NS

a = Least Significant Range (Appendix C)

S = significant at $P < .05$

NS = not significant at $P > .05$

Shelf-lives of all various nifedipine gels were predicted, based on first-order kinetics, and presented in Table 10. The shelf-life of nifedipine gel wrapped in aluminium foil (formulation Ic) could not be predicted because it was stable throughout this study. Results in Table 11 showed a statistically significant difference between nifedipine gel exposed to normal light (formulation Ib) and nifedipine gel exposed to accelerated light (formulation Ia) ($P < .05$). Tables 12 and 13 showed statistically siginificant differences among formulations Ia, II, III, IV and V ($P < .05$). However, there was no statistically significant difference between formulations IV and V ($P > .05$).

In this study, the results of degradation rate constants and shelf-lives indicated that (a) addition of sodium bisulfite as the antioxidant in nifedipine gels exposed to accelerated light could reduce photodegradation of nifedipine gel and the antioxidative efficacy of sodium bisulfite could be ranked according to its concentration as follows : 0.30 and 0.50 > 0.10 > 0.05 > 0.00 % w/w ($P < .05$). This might be due to the property of being reducing agent of sodium bisulfite. (b) The predicted shelf-life of nifedipine gel formulation under normal light was shown to be much longer than that under accelerated light. (c) Aluminium foil could prevent

Table 10 Shelf-lives of Seven Nifedipine Gels

Formulations	Shelf-lives (Hours)[a]
Ia	1.104 ± 0.072
Ib	11.032 ± 0.233
Ic	∞
II	1.272 ± 0.024
III	1.960 ± 0.037
IV	2.840 ± 0.055
V	2.784 ± 0.024

a = Mean ± S.D. (n = 3)

Table 11 Comparison of Shelf-lives of Nifedipine Gel Under Normal Light with Nifedipine Gel Under Accelerated Light, Using Student's t-test.

Formulation	t-value (calculated) Comparison with Nifedipine Gel Under Accelerated Light	Statistical Significance
Nifedipine Gel Under Normal Light	- 70.914	S

$$t^{a}_{(.05,\ 4)} = 2.776$$

S = significant at $P < .05$

a = t-value from the table

Table 12 Analysis of Variance for Shelf-lives of Nifedipine Gels with Various Concentrations of Sodium Bisulfite

Source of Variation	df[a]	SS[b]	MS[c]	F[d]
Among Groups	4	7.963	1.9908	948.00
Within Group	10	0.021	0.0021	
Total	14	7.984		

$$F^{e}_{.05(4,\ 10)} = 3.48$$

a = degree of freedom

b = Sum of Square

c = Mean Square

d = Variance Ratio

e = F-value obtained from the table

Table 13 Comparison of Shelf-lives of Nifedipine Gels with Various Concentrations of Sodium Bisulfite, Using Duncan's New Multiple Range Test.

Formulations	Difference Between Means	LSR[a]	Statistical Significance
Ia versus II	0.168	0.083	S
Ia versus III	0.856	0.087	S
Ia versus IV	1.736	0.091	S
Ia versus V	1.680	0.089	S
II versus III	0.688	0.083	S
II versus IV	1.568	0.089	S
II versus V	1.512	0.087	S
III versus IV	0.880	0.087	S
III versus V	0.824	0.083	S
IV versus V	0.056	0.083	NS

a = Least Significant Range (Appendix C)

S = significant at $P < .05$

NS = not significant at $P > .05$

photodegradation of nifedipine gel. This might be due to the protective property from light of aluminium foil. Tucker, Minty and MacGregor (1985) also found that nifedipine in whole blood or plasma wrapped in foil showed no reduction in nifedipine concentration. Another study (Al-Turk et al., 1988) also showed that amber glass bottles could prevent degradation of nifedipine when exposed to light.

In addition, the effect of concentration of sodium bisulfite on the degradation of nifedipine gel was shown in Table 14 and Figure 9. The higher the concentration of sodium bisulfite was, the lower the degradation rate constant obtained. However, the decrease in the degradation rate constant was limited to 0.30% w/w of sodium bisulfite. As shown in Table 9, there was no significant difference between degradation rate constants of the formulations containing 0.30 and 0.50% w/w of sodium bisulfite. Correlation between the concentration of sodium bisulfite (0.00-0.30% w/w) and the degradation rate constant of nifedipine gel was determined in Table 15. It indicated that there was significant correlation between the concentration of sodium bisulfite and the degradation rate constant of nifedipine gel ($P < .10$). According to this correlation, the increase in the photostability of nifedipine gel was

Table 14 Degradation Rate Constants of Nifedipine Gels as a Function of Sodium Bisulfite Concentrations

Sodium Bisulfite Concentrations (% w/w)	Degradation Rate Constants ($Days^{-1}$)[a]
0.00	2.2895 ± 0.1425
0.05	1.9777 ± 0.0458
0.10	1.2824 ± 0.0223
0.30	0.8867 ± 0.0189
0.50	0.9059 ± 0.0101

a = Mean ± S.D. (n = 3)

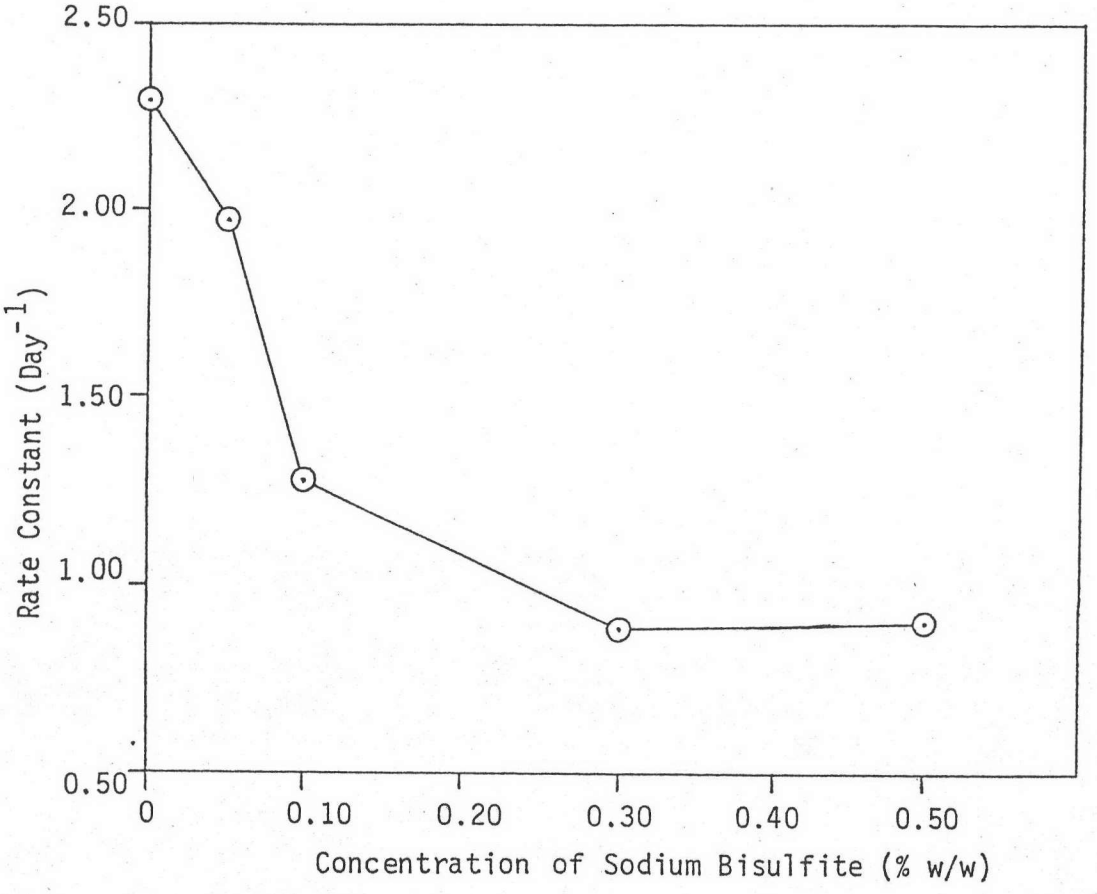

Figure 9 Effect of Concentration of Sodium Bisulfite on the Degradation of Nifedipine Gel. Plot of Rate Constant versus Concentrations of Sodium Bisulfite

Table 15 Concentration of Sodium Bisulfite-Degradation Rate Constant of Nifedipine Gel Correlation

Correlation	df[a]	Correlation Coefficient	t-value (calculated)	Statistical Significance
Conc. of Sodium Bisulfite versus Degradation Rate Constants	2	- 0.915	3.212	S

$$t^{b}_{(.10,\ 2)} = 2.920$$

a = degree of freedom = number of pairs-2

S = significant at P < .10

b = t-value from the table

directly proportional to the concentration of sodium bisulfite.

From the results of this study, the method which could virtually prevent photodegradation of nifedipine gel was to protect the preparation from light by wrapping in aluminium foil and this seemed to be a suitable packaging for nifedipine gel.