CHAPTER IV

RESULTS AND DISCUSSION

Stability Indicating Assay

A scan of indomethacin in hydroalcoholic solution by the UV spectrophotometer was compared to that of its degraded product and is shown in figure 8. It indicated that the degraded product has almost no effect on the UV absorption of indomethacin at the wavelength of 318 nm.

Calibration Curve Determination

The calibration data for analyzing indomethacin concentrations in solution and gel preparations are shown in tables 4 and 5, respectively. Their corresponding calibration curves are shown in figures 9 and 10, respectively. The regression lines obtained is "absorbance = 0.0033 + (0.1745 x concentration)" for indomethacin solutions and is "absorbance = 0.1396 + (0.1689 x concentration)" for indomethacin gels. The coefficients of determination (r^2) of both regression lines are highly significant ($r^2 = 0.999$). Indomethacin concentrations estimated from the regression lines are called "inversely estimated concentrations". The "inversely estimated concentrations" were calculated to percentage by comparing them with their corresponding actual concentrations. These

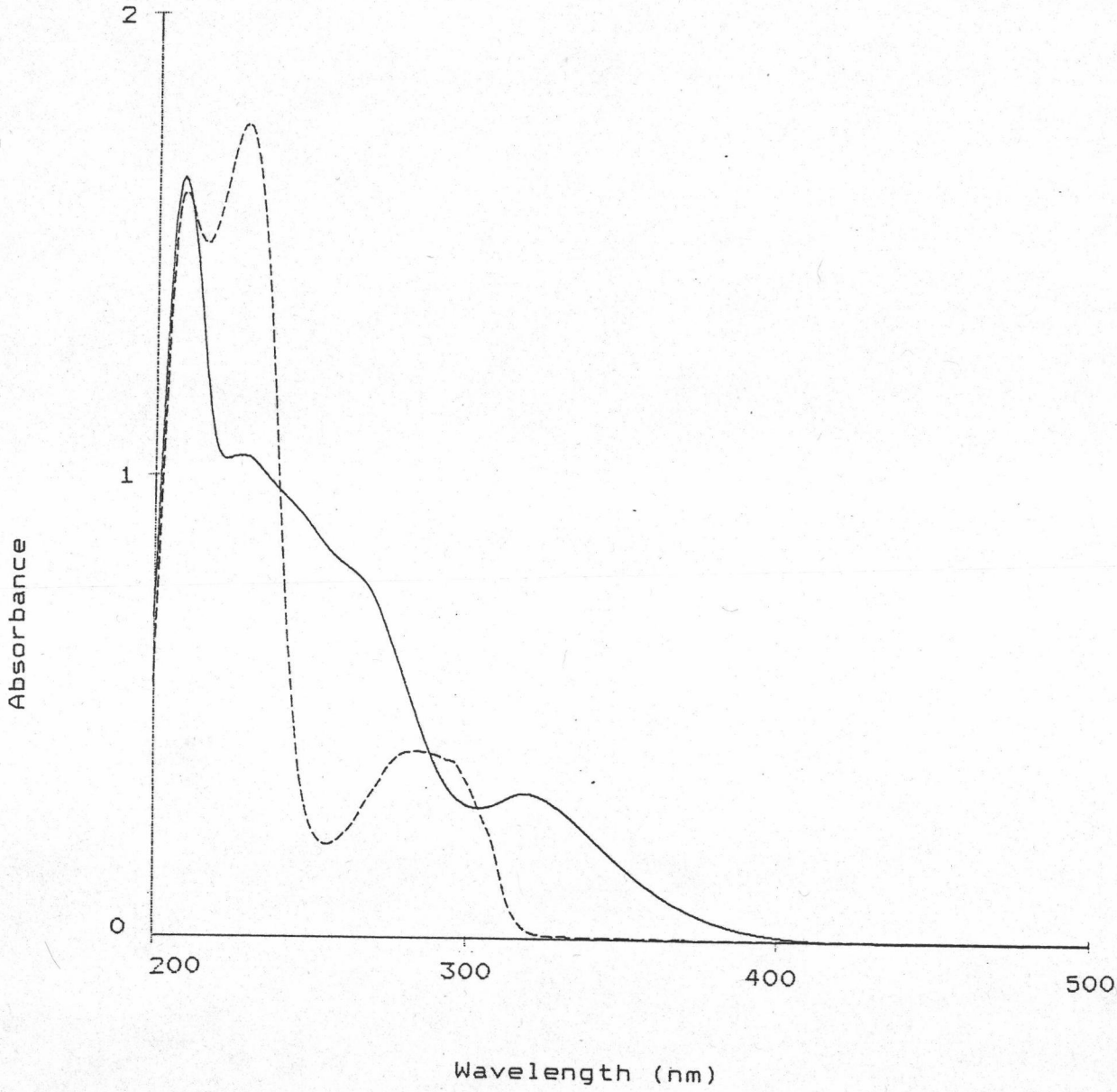

Figure 8. The scans of indomethacin solution (———) and its degraded product (------).

Table 4 : Calibration curve data of indomethacin solutions

Std.No.	conc. (mg %)	absorbance	inversely estimated conc.[a]	% theory[b]
1	0.599	0.109	0.606	101.169
2	1.198	0.214	1.207	100.751
3	1.797	0.315	1.786	99.388
4	2.396	0.419	2.382	99.416
5	2.995	0.524	2.984	99.633
6	3.594	0.630	3.591	99.917
7	4.193	0.742	4.233	100.954
8	4.792	0.836	4.772	99.583
				Mean 100.101
				S.D. 0.736
				C.V.[c] 0.735

[a] obtained from the fitted curve:

absorbance = 0.0033 + (0.1745 x conc.); $R^2 = 0.999$

Inversely estimated concentration = $\frac{(\text{Absorbance}-0.0033)}{0.1745}$

[b] % Theory = $\frac{\text{Inversely estimated concentration} \times 100}{\text{Known concentration}}$

[c] Coefficient of variation = $\frac{\text{S.D.} \times 100}{\text{Mean}}$

Table 5 : Calibration curve data of indomethacin gels

Std.No.	conc. (mg %)	absorbance	inversely estimated conc.[a]	% theory[b]
1	0.599	0.244	0.617	102.941
2	1.198	0.342	1.198	100.018
3	1.797	0.446	1.815	101.026
4	2.396	0.541	2.379	99.300
5	2.995	0.645	2.996	100.048
6	3.594	0.740	3.560	99.061
7	4.193	0.846	4.189	99.912
8	4.792	0.957	4.848	101.170
				Mean 100.393
				S.D. 1.154
				C.V.[c] 1.149

a obtained from the fitted curve:

$$\text{absorbance} = 0.1396 + (0.1689 \times \text{conc.}); \quad R^2 = 0.999$$

$$\text{Inversely estimated concentration} = \frac{\text{Absorbance} - 0.1369}{0.1689}$$

b

$$\% \text{ Theory} = \frac{\text{Inversely estimated concentration} \times 100}{\text{Known concentration}}$$

c

$$\text{coefficient of variation} = \frac{\text{S.D.} \times 100}{\text{Mean}}$$

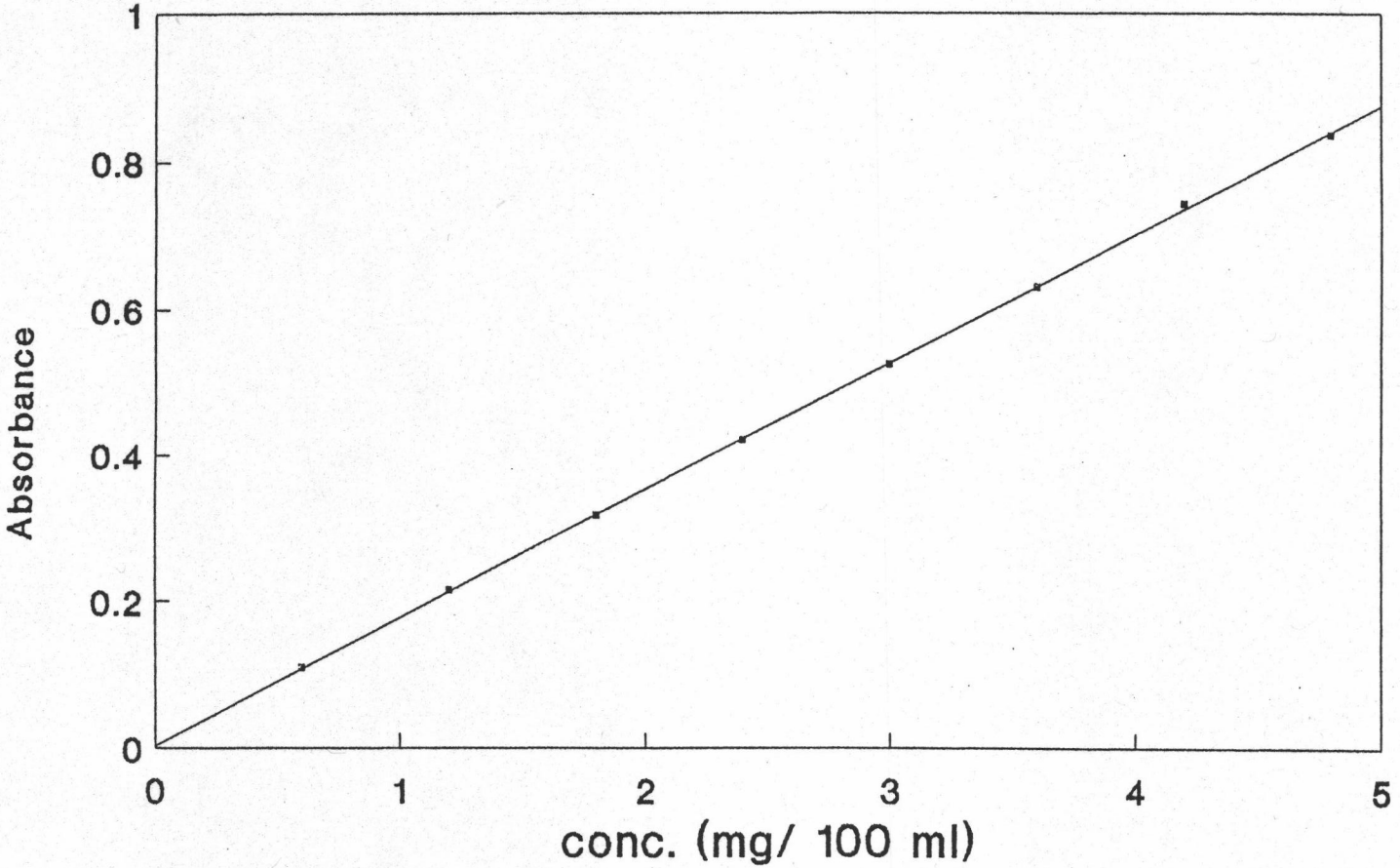

Figure 9. A calibration curve of indomethacin solutions.

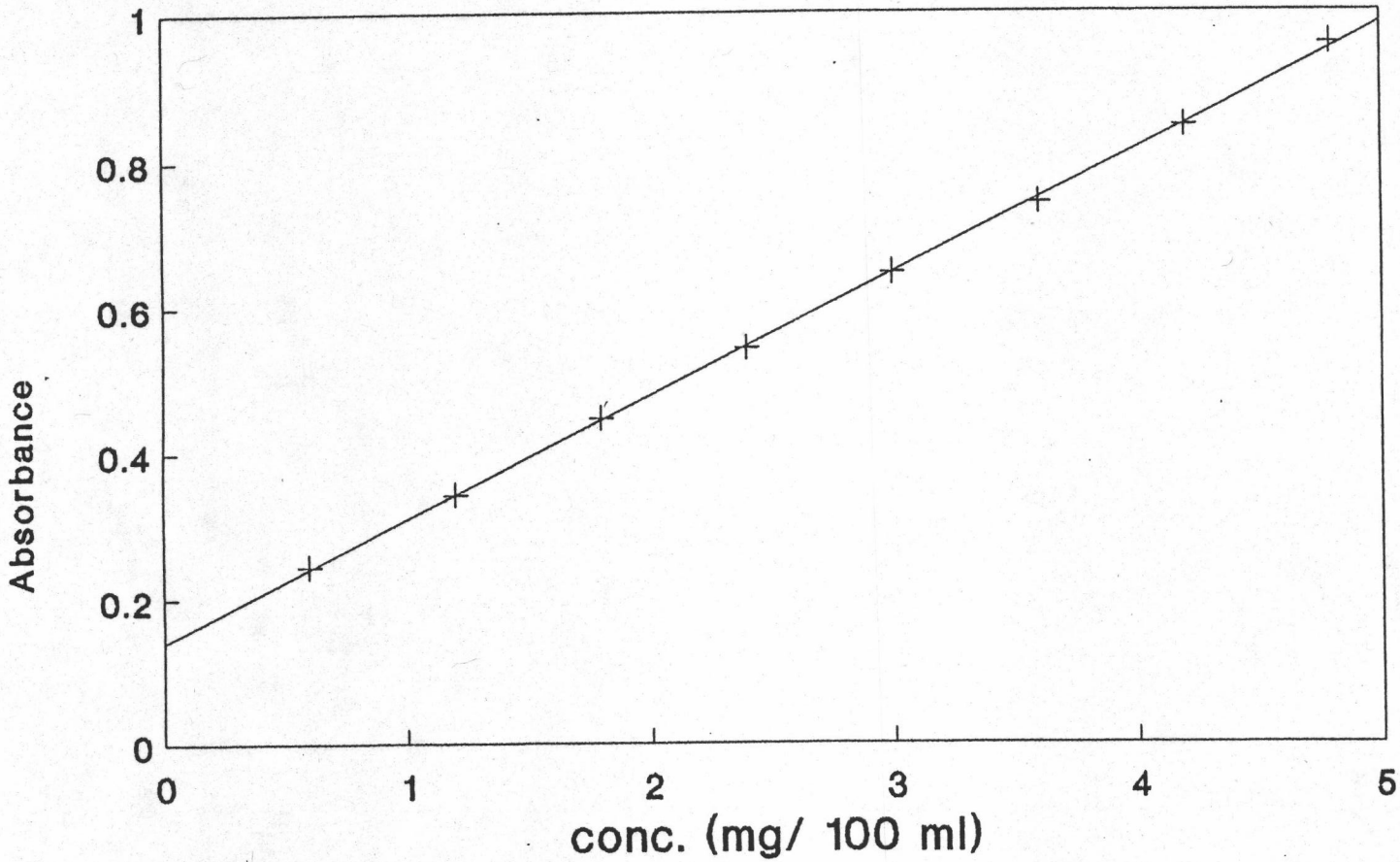

Figure 10. A calibration curve of indomethacin gels.

percentages of the inversely estimated concentrations are so called "percent theories". The coefficient of variation of the percent theories was determined from their mean and standard deviation. It is a parameter which indicates the variation of the variables from the fitted line. The coefficient of variations of both data which are much less than 2 % are highly accepted.

Formulation of Topical Indomethacin Solutions

Solubilization of Indomethacin

In general, a formulation of a topical solution is composed of a dissolved drug or drugs in a suitable vehicle containing other ingredients as needed, e.g., preservative, humectant or emollient, colour, odour, etc.. Actually, an aqueous solution is most suitable for pharmaceutical prepations because water is the most safety vehicle. Indomethacin is soluble in an alkaline solution but with decomposition. So other solvents were selected.

There had been many studies of indomethacin solubilities as stated in Chapter II. From these studies acetone was the best solubilizer of indomethacin but it is not a good solvent for medicated preparations because of its damaging effect on the permeability barrier of the skin (Bond and Barry, 1988). The appropriate solvents for the medicated preparations and for indomethacin are ethanol and propylene glycol. Ethanol has been used as a solvent for topical preparations for a long time, e.g.,

for medicinal tincture for application. However, a long term use of alcohol to human skin may cause dryness of the skin by dehydration action of alcohol, resulting in decreasing percutaneous penetration of the drug. Propylene glycol has been widely used as a solvent for topical preparations. As propylene glycol is also a humectant, it increases stratum corneum hydration, resulting in an increase in the penetration rate across the skin. Although the use of propylene glycol improves the drug solubility and bioavailability in topical formulations, propylene glycol had been reported to cause irritation and/or sensitization when its concentration exceeds ten percent (Narter, Baar and Hoedemaeder, 1977).

Solubilities of indomethacin in various solvent mixtures are presented in table 6. Although the solvent mixtures of 20 % propylene glycol and 60 % or 70 % alcohol (solvent mixtures No. 1 and 5) are good solubilizers of indomethacin as shown in table 6, they may cause irritation as propylene glycol content exceeds ten percent. Solvent mixtures No. 2 was excluded as indomethacin solubility is too low. Therefore, solvent mixtures No. 3, 4, 6, 7 and 8 would be considered. Solvent mixtures No. 6, 7 and 8 have more alcoholic content than solvent mixtures No. 3 and 4. To avoid the effect of dehydration of alcohol, the less ones were chosen, i.e., solvent mixtures No. 3 and 4 were accepted for furthur study. Solvent mixture No. 3 is more interesting than

Table 6 : Solubilities of indomethacin in solvent mixtures

Solvent Mixture No.	Alcohol (% v/v)	Propylene glycol (% v/v)	Solubility of indomethacin (g %)
1	60	20.0	1.532
2	70	5.0	0.770
3	70	7.5	0.998
4	70	10.0	1.011
5	70	20.0	1.864
6	80	5.0	1.264
7	80	7.5	1.415
8	80	10.0	1.420

solvent mixture No. 4 because propylene glycol content of the latter is nearly equal to its maximum non-irritant content. Therefore, the sovent mixture No. 3 is appropriate for indomethacin preparation. However, the solubility of indomethacin in solvent mixture No. 3 is not enough to use as a topical solution (1 g %).

To increase the indomethacin solubility, other solubilizers were needed. Some surfactants had been reported to increase indomethacin solubilities (Lin and Kawashima, 1985; Krasowska, 1979; Tomida, Kuwada and Kiryu, 1988). These include pluronic F127, pluronic F68 and polysorbate 80. Besides being solubilizers, they were expected to help stabilizing the drug. The indomethacin solubilities in solvent mixtures No. 3 containing these three surfactants are reported in table 7. The solubilities of indomethacin in all solvent mixtures containing the three surfactants are much more than 1 g/100 ml. Note that 5 % surfactants are greater than their critical micelle concentration. Consequently, the solvent mixtures of 70 % ethanol, 7.5 % propylene glycol and 5 % surfactant were chosen for further study. Other ingredients, e.g., a preservative and a humectant are not neccessary here because alcohol can preserve when its concentration exceeds 20 % (Rawlins, 1977) and propylene glycol in the formulations can act as a humectant. Furthermore, a colour and an odour were not studied because they may complicate the system and they are not

Table 7 : Solubilities of indomethacin in solvent mixtures No. 3 including the surfactants

Solvent Mixture No.	Polysorbate 80 (g %)	Pluronic F127 (g %)	Pluronic F68 (g %)	Solubility of indomethacin (g %)
9	5	-	-	1.512
10	-	5	-	1.346
11	-	-	5	1.426

neccessary here.

Stability Analysis

Using the solublity data, topical indomethacin solutions were formulated and their details are shown in table 8.

To investigate whether increased amount of the surfactants could increase the stability of indomethacin, two concentrations of the surfactants were studied, i.e., 5 % and 10 % (formulation No. 1-6). Formulation No. 7 was performed to examine whether the surfactants help stabilizing the systems. Since there is no surfactant in this formulation, 1 g % of indomethacin could not dissolve and therefore, 0.6 g % of indomethacin was prepared for this examination. According to Hajratwala and Dawson (1977), the kinetics of indomethacin degradation followed first order kinetics, so the initial concentrations do not affect the half life and/or shelf life of indomethacin.

As stated in Chapter II that the hydrolysis of indomethacin is first order kinetic and can be accelerated by elevated temperatures. The elevated temperature selected for preliminary study of the stabilities of these preparations was 70°C because it is high enough to accelerate the drug decomposition rate, yet it does not exceed the boiling point of ethanol which is 78°C. From this study, the most stable formulation would be selected for stability study at other elevated temperatures. How

Table 8 : Formulations of prepared topical indomethacin solutions

Formulation No.	Alcohol (% v/v)	Propylene glycol (% v/v)	Polysorbate 80 (% w/v)	Pluronic F127 (% w/v)	Pluronic F68 (% w/v)
1	70	7.5	5	-	-
2	70	7.5	10	-	-
3	70	7.5	-	5	-
4	70	7.5	-	10	-
5	70	7.5	-	-	5
6	70	7.5	-	-	10
7[a]	70	7.5	-	-	-

[a] This formulation contained 0.6 g % of indomethacin.

stable a formulation was was considered from its rate constant which was a slope of a concentration vs time plot for zero-order kinetics or a slope of log (concentration) vs time plot for first-order kinetics. These plots also indicate the decomposition kinetics of indomethacin. Rate constants of all seven preparations performed at 70°C are shown in table 9. The corresponding concentration-time profiles and log (concentration)-time profiles are shown in figures 11 and 12, respectively. According to table 9, the zero-order rate constant of formulation No.7 is the lowest. This is because the zero-order degradation rate constant depends on the initial concentration of the drug (Connor, Amidon and Kennon, 1979). Therefore, the first-order rate constants would be used for the comparison of their stabilities. As can be seen that the first order rate constant of formulation No. 7 is equal to that of formulation No. 5 and is close to formulation No. 1. Therefore, it could be concluded that the surfactants do not help stabilizing the drug. In other words, they only are solubilizers for these systems. In addition, increasing the surfactant concentrations increase the degradation rates. An increase in alcoholic content would lower the concentration of hydroxide ions and, therefore, the protection role of micelles in this system was less important than the system of less alcoholic content. Furthermore, indomethacin hydrolysis occurs by the attack of hydroxide ions on which solvent polarity has

Table 9 : The rate constants (k) of the prepared topical indomethacin solutions at 70°C

Formulation No.	k_0 ($mg\ day^{-1}$)[a]	$k_1 \times 10^3$ (day^{-1})[b]
1	1.278 ± 0.115[c]	1.421 ± 0.131[c]
2	1.640 ± 0.081[c]	1.864 ± 0.086[c]
3	1.416 ± 0.130[c]	1.528 ± 0.142[c]
4	1.622 ± 0.115[c]	1.869 ± 0.142[c]
5	1.286 ± 0.113[c]	1.407 ± 0.119[c]
6	1.466 ± 0.079[c]	1.624 ± 0.086[c]
7	0.785 ± 0.084[c]	1.407 ± 0.086[c]

a k_0 was obtained from the slope of concentration vs time curve (zero-order degradation).

b k_1 was obtained from the slope of ln (concentration) vs time curve (first-order degradation).

c 95 % confidence limit of rate constant is obtained from $b \pm t(d.f.,0.95) \times Sb$, where b is the slope or rate constant and Sb is the variance of the estimate of the rate constant.

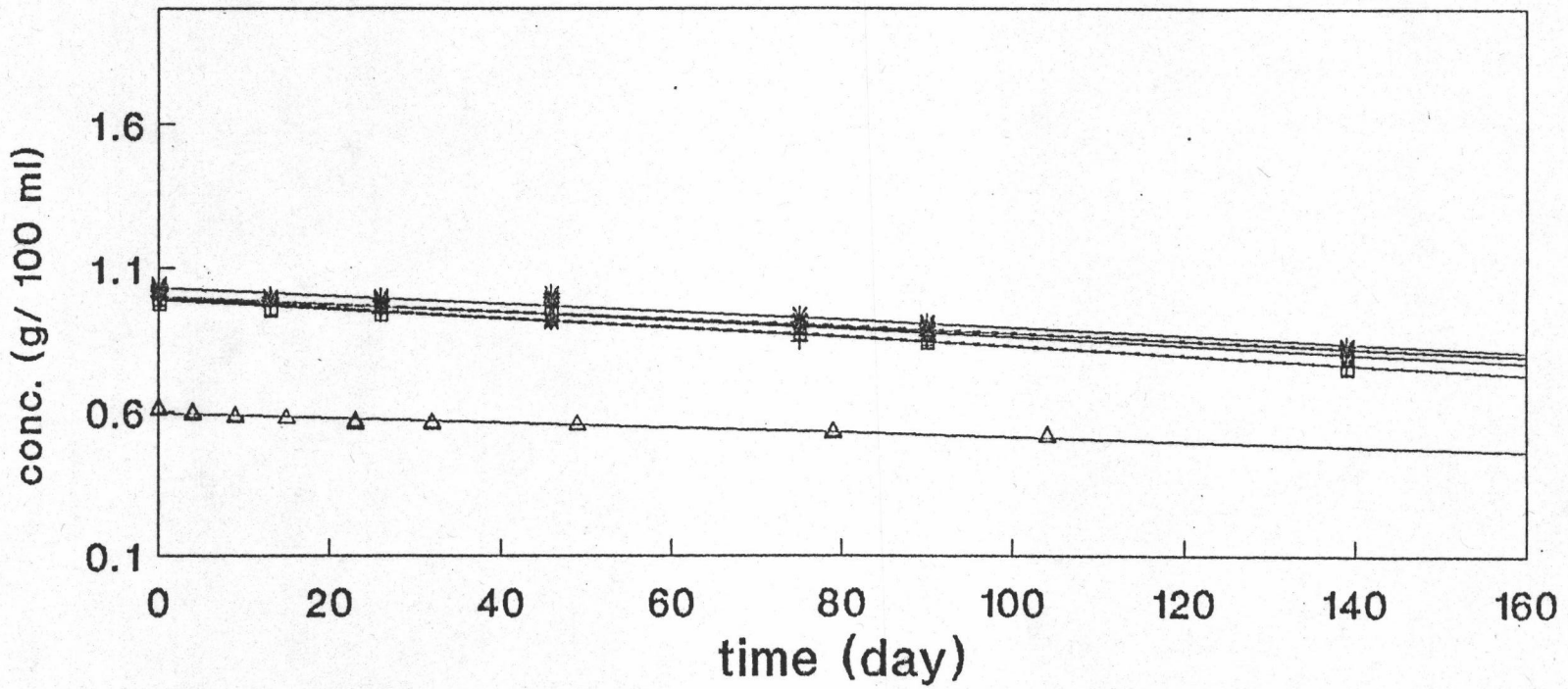

Figure 11. The concentration vs time plots of the prepared indomethacin solutions at 70°C (Formulation No. 1,—•—; Formulation No. 2,—|—; Formulation No. 3,—✳—; Formulation No. 4,—▯—; Formulation No. 5,—×—; Formulation NO. 6,—◇—; Formulation No. 7, —△—).

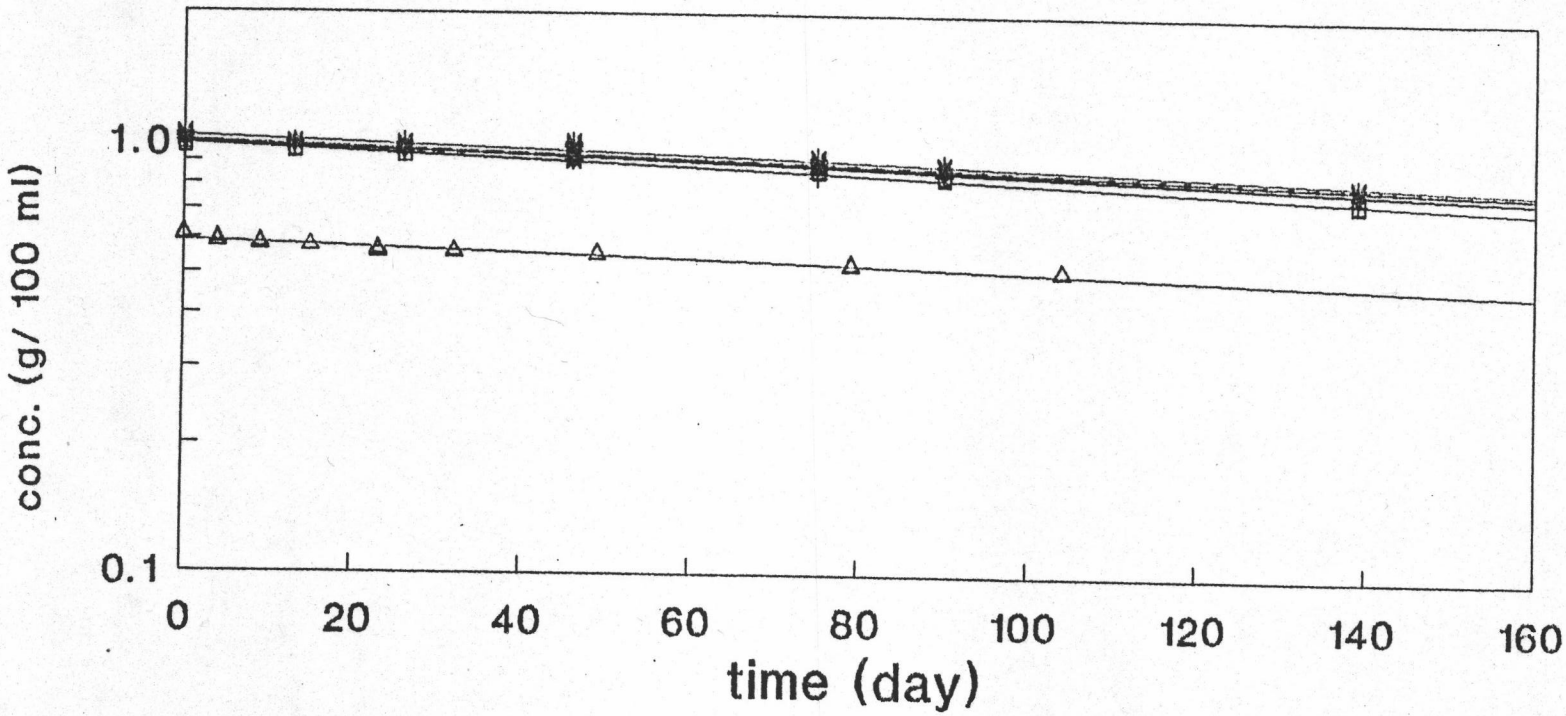

Figure 12. The semilog plots of concentration vs time of the prepared indomethacin solutions at 70 °C (Formulation No. 1, —•—; Formulation No. 2, —+—; Formulation No. 3, —✳— ; Formulation No. 4, —□—; Formulation No. 5, —×— ; Formulation NO. 6, —◇— ; Formulation No. 7, —△—).

much influence. Decrease in solvent polarity is expected to decrease the rate of reaction (Connor, Amidon and Kennon, 1979). The dielectric constant of alcohol is less than that of water, therefore, the rate of indomethacin hydrolysis decreses in the systems of high alcoholic content. Higher concentrations of the surfactants might increase the dielectric constant of the solutions and therefore, increase the rate constants. The rate constants of formulation No. 1, 3 and 5 are comparable. Thus, instead of the most stable formulation these three formulations would be studied furthur.

Rate constants of the three preparations at 40°C, 50°C, 60°C and 70°C are shown in table 10. The corresponding concentration-time profiles (zero-order kinetics) and log (concentration)-time profiles (first-order kinetics) are shown in figures 13-18.

The comparison of correlation coefficient between concentration-time and log (concentration)-time curves might help indicating the order of reaction. In table 11, the pairs of coefficient of determination of the zero-order and first-order rate constants are not quite different. This is possible because the degradations are very little. Though indomethacin degradation is known to be first order, this study cannot indicate which order the reaction is. So the estimates of both zero and first order reactions are reported.

Table 10 : The rate constants (k) of the prepared topical indomethacin solutions at 40°C, 50°C, 60°C and 70°C

Formulation No.	Temp (°C)	k_0 (mg day^{-1})[a]	$k_1 \times 10^3$ (day^{-1})[b]
1	40	0.105± 0.022[c]	0.103± 0.021[c]
	50	0.125± 0.036[c]	0.122± 0.036[c]
	60	0.305± 0.092[c]	0.301± 0.090[c]
	70	1.278± 0.115[c]	1.421± 0.131[c]
3	40	0.088± 0.024[c]	0.085± 0.023[c]
	50	0.257± 0.038[c]	0.250± 0.037[c]
	60	0.516± 0.134[c]	0.511± 0.134[c]
	70	1.416± 0.130[c]	1.528± 0.142[c]
5	40	0.128± 0.035[c]	0.124± 0.034[c]
	50	0.238± 0.039[c]	0.023± 0.039[c]
	60	0.472± 0.092[c]	0.460± 0.090[c]
	70	1.286± 0.113[c]	1.407± 0.119[c]

[a] k_0 was obtained from the slope of concentration vs time curve (zero-order degradation).

[b] k_1 was obtained from the slope of ln (concentration) vs time curve (first-order degradation).

[c] The 95 % confidence limit of the rate constant is obtained from $b \pm t(d.f.,0.95) \times Sb$, where b is the rate constant and Sb is the variance of the estimate of the rate constant.

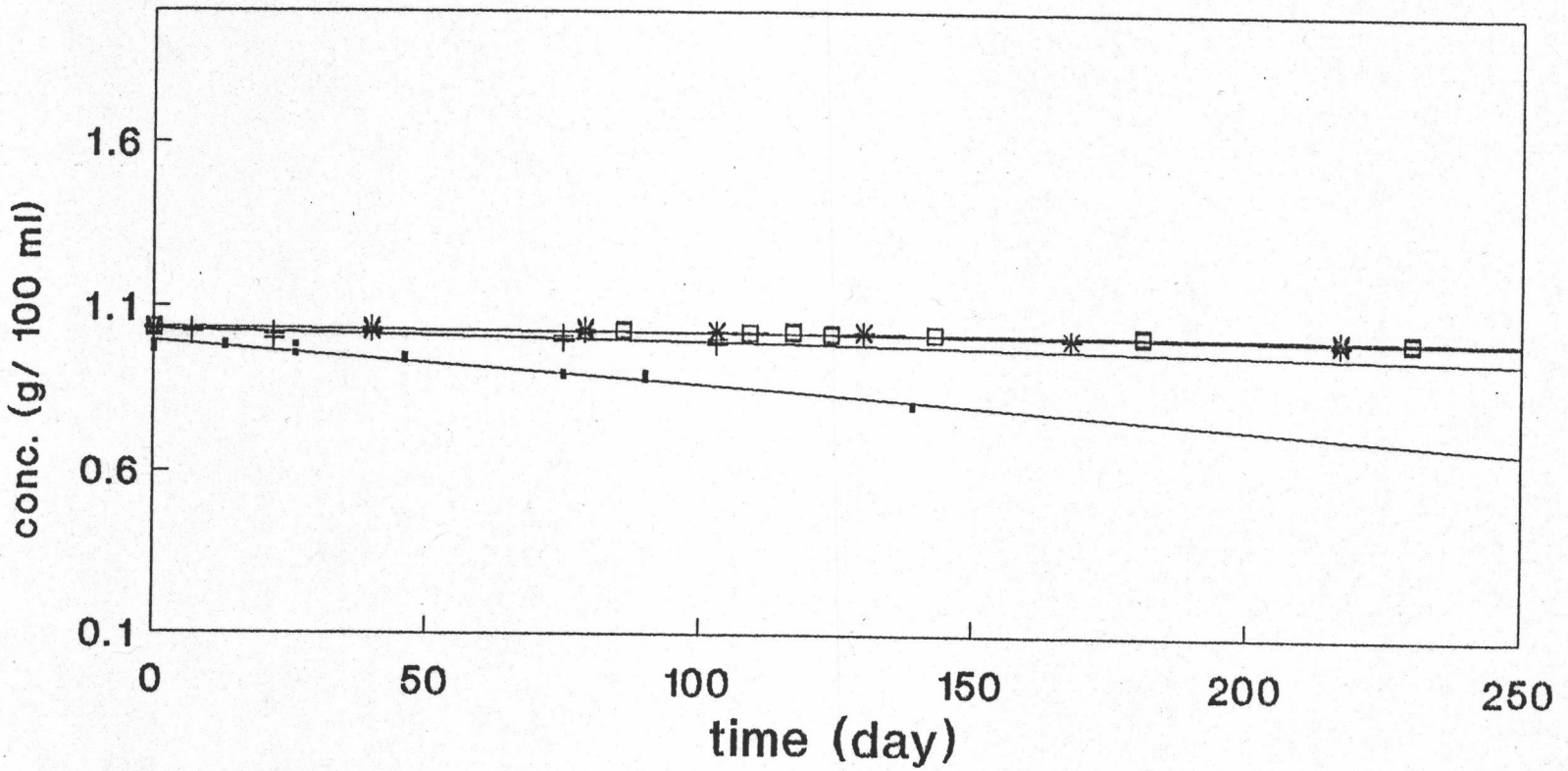

Figure 13. The concentration vs time plots of formulation No. 1 (containing polysorbate 80) at elevated temperatures (70°C, —•— ; 60°C, —+— ; 50°C, —✳— ; 40°C, —□—).

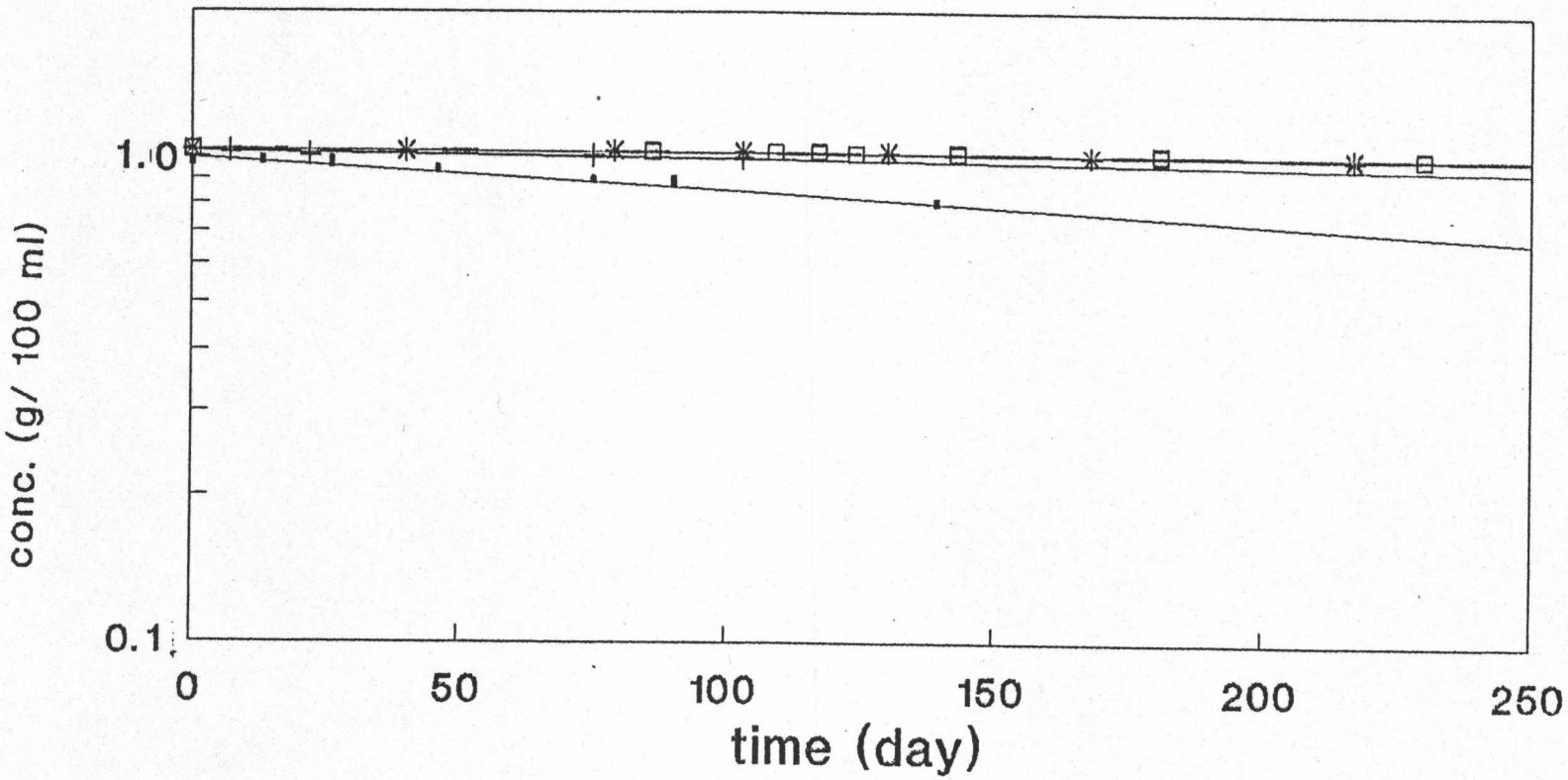

Figure 14. The semilog plots of concentration vs time of formulation No. 1 (containing polysorbate 80) at elevated temperatures (70°C, —•— ; 60°C, —|— ; 50°C, —*— ; 40°C, —□—).

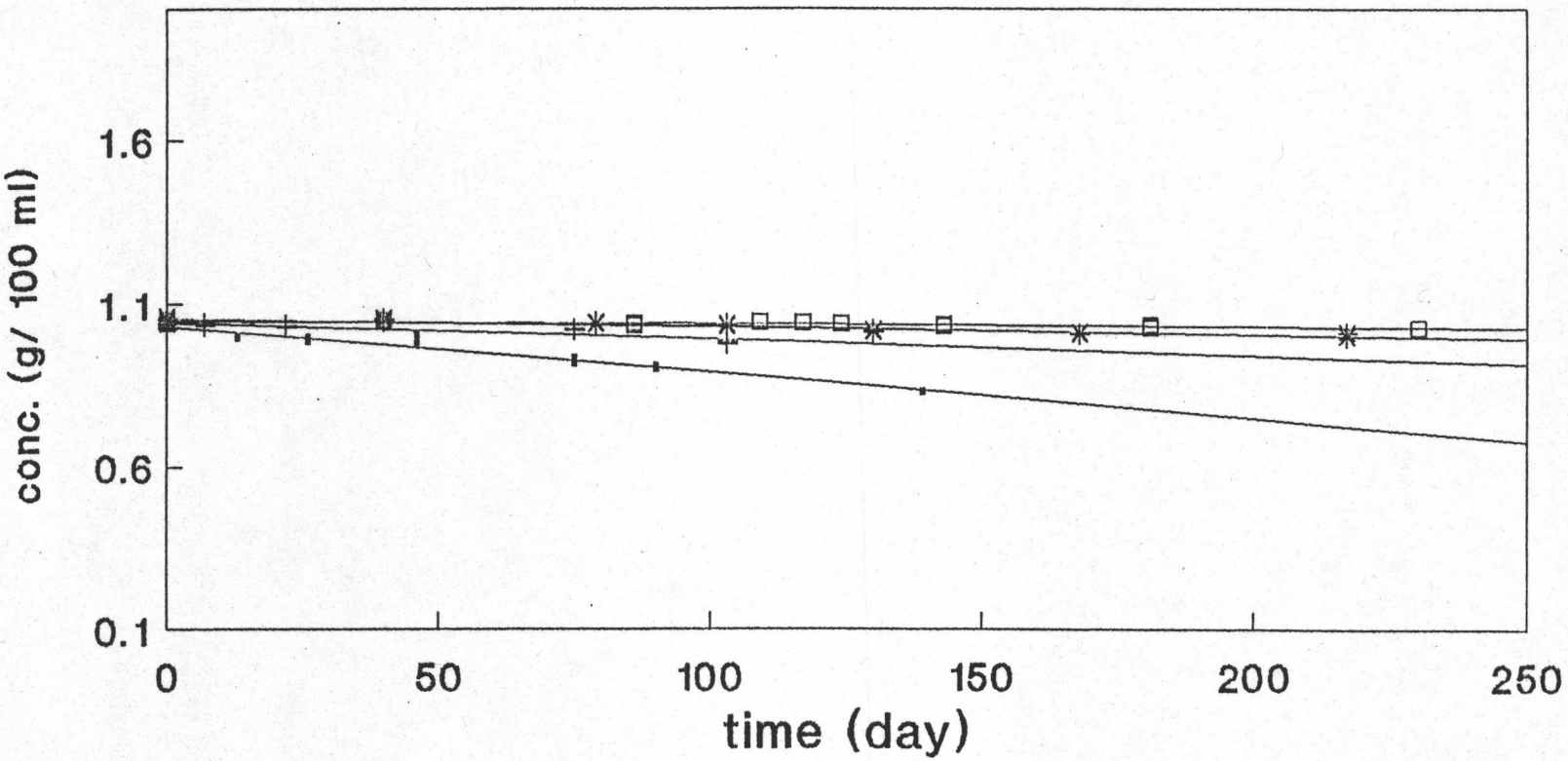

Figure 15. The concentration vs time plots of formulation No. 3 (containing pluronic F127) at elevated temperatures (70 °C, —·—; 60 °C, —+—; 50 °C, —*— ; 40 °C, —□—).

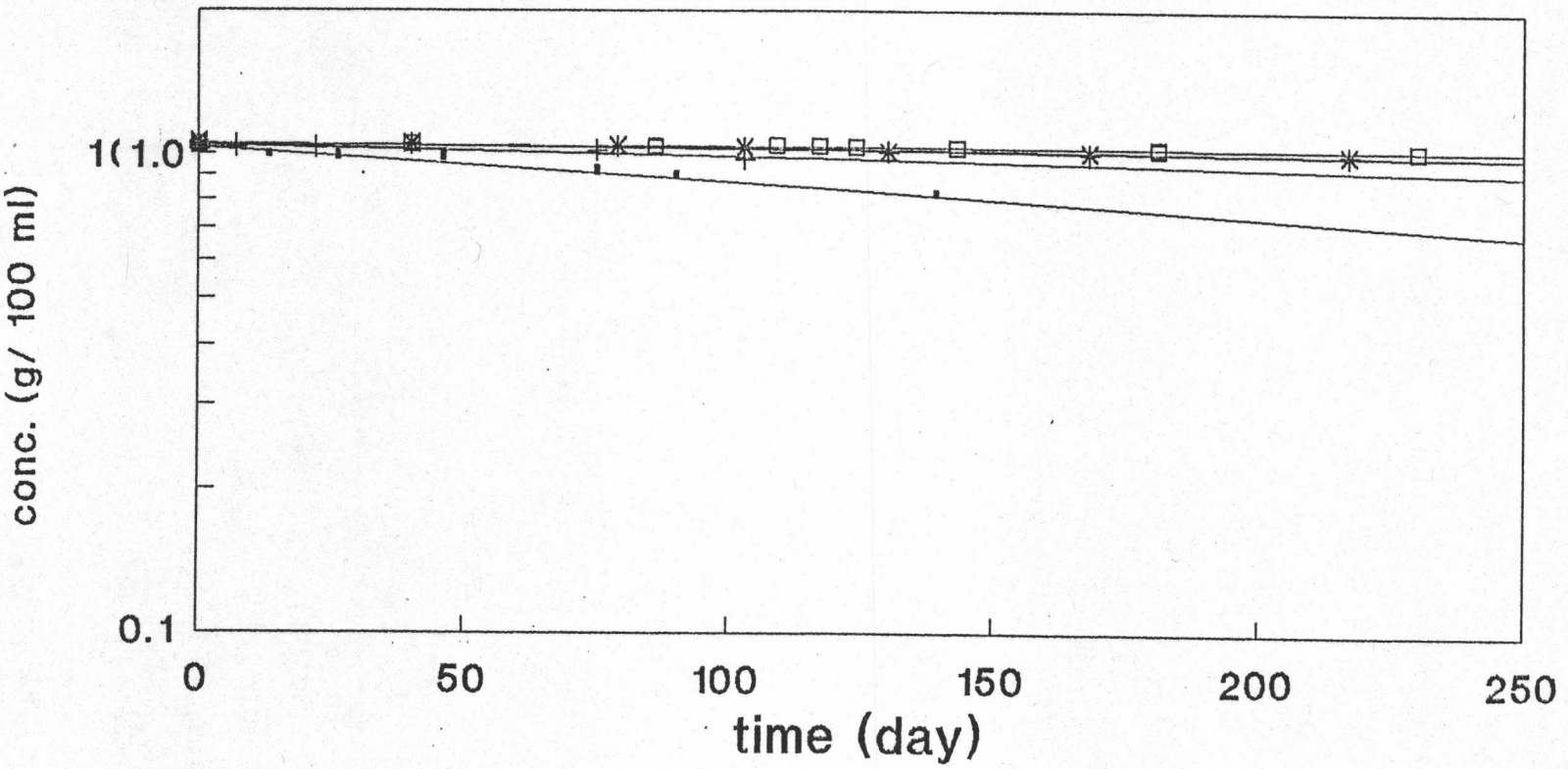

Figure 16. The semilog plots of concentration vs time of formulation No. 3 (containing pluronic F127) at elevated temperatures (70°C, —•—; 60°C, —+—; 50°C, —*—; 40°C, —□—).

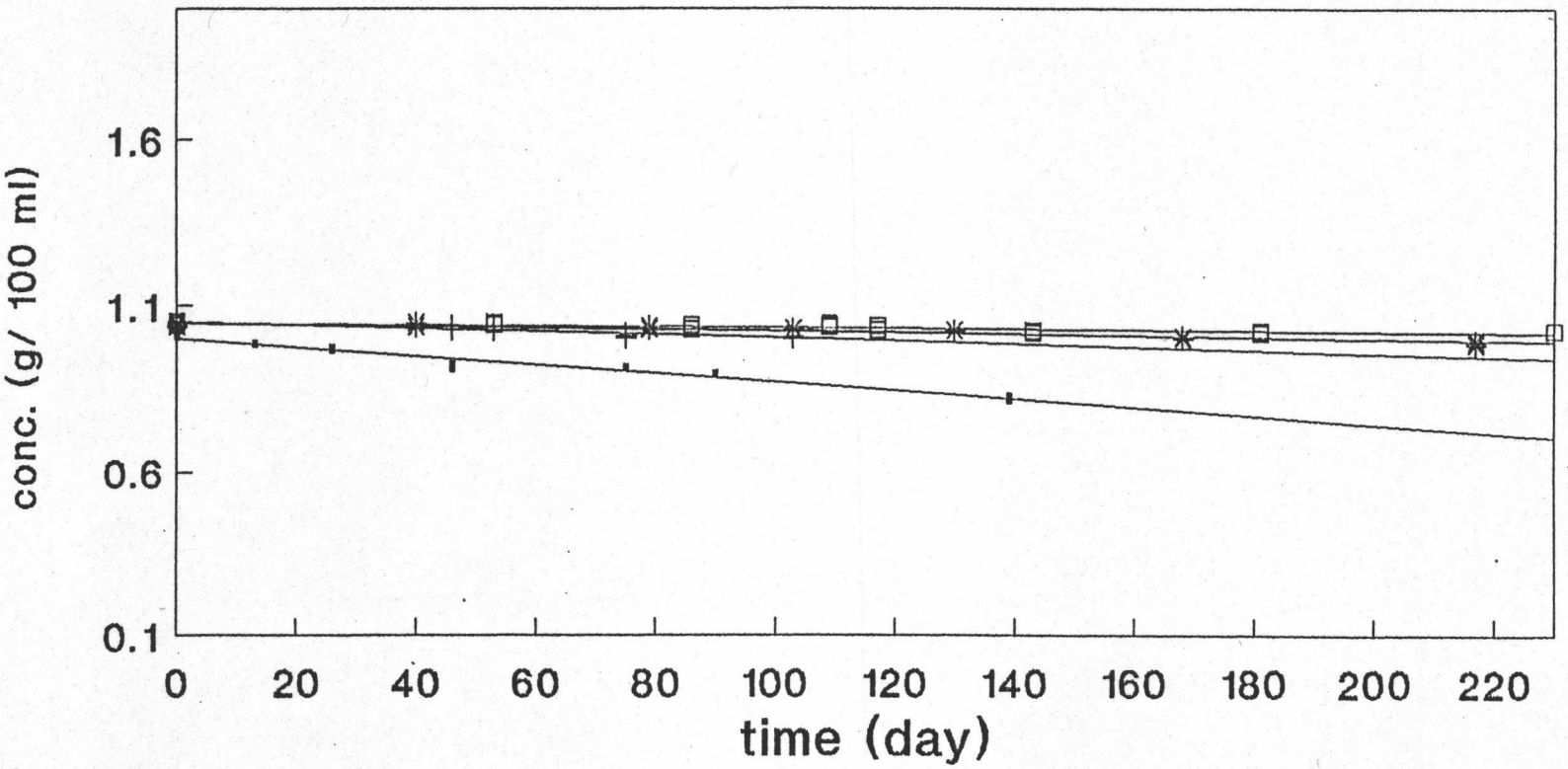

Figure 17. The concentration vs time plots of formulation No. 5 (containing pluronic F68) at elevated temperatures (70°C, —•— ; 60°C,—|— ; 50°C, —✳— ; 40°C, —□—).

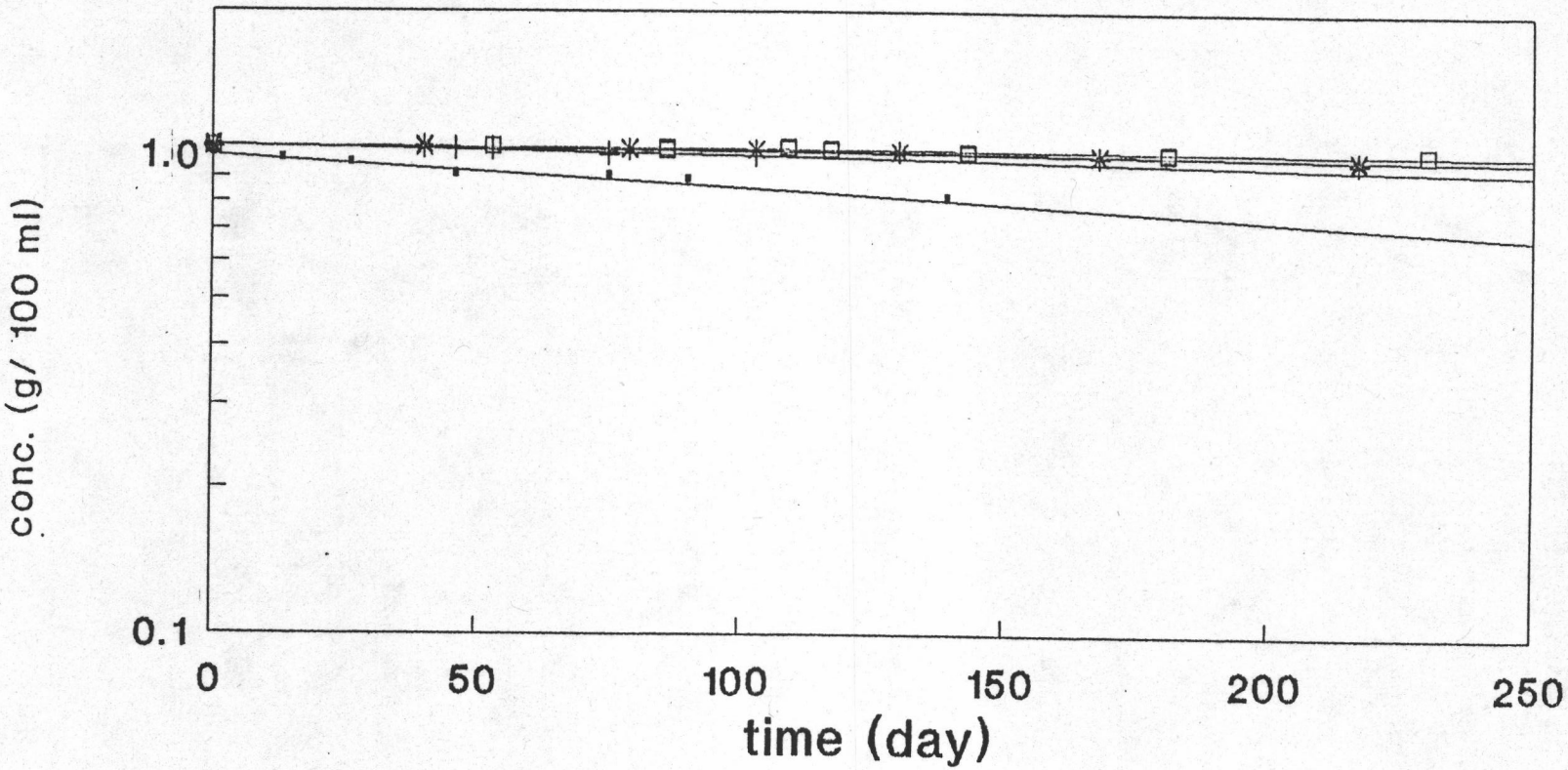

Figure 18. The semilog plots of concentration vs time of formulation No. 5 (containing pluronic F68) at elevated temperatures (70°C, —•—; 60°C, —|—; 50°C, —✳—; 40°C, —□—).

Table 11 : The correlation coefficient obtained from the concentration vs time profiles and the log (concentration) vs time profiles of formulation No. 1, 3, and 5

Formulation No.	Temp (°C)	zero-order	first-order
		r	r
1	40	0.8759	0.8755
	50	0.8116	0.8114
	60	0.8559	0.8569
	70	0.9760	0.9740
3	40	0.8116	0.8116
	50	0.9378	0.9383
	60	0.8858	0.8848
	70	0.9751	0.9744
5	40	0.8097	0.8091
	50	0.9261	0.9248
	60	0.9306	0.9302
	70	0.9772	0.9785

Arrhenius Plot of the Prepared Topical Indomethacin Solutions

The Arrhenius plots of prepared topical indomethacin solutions were performed (figure 19-20) using the Arrhenius equation:

$$\log k = \log A - \frac{Ea}{2.303\,RT} \quad (15)$$

where k is the rate constant, A is the frequency factor, Ea is the activation energy, and R is the gas constant (1.987 cal mol^{-1} K^{-1}), and T is the absolute temperature. These estimates, Ea and log A, are shown in table 12. The rate constants at any temperatures can be estimated using the Arrhenius equation and the corresponding shelf lives ($t_{0.9}$) can be calculated as follows:

$$t_{0.9} = \frac{0.105}{k_1} \quad (9)$$

for first-order kinetics and

$$t_{0.9} = \frac{0.1[D]}{k_0} \quad (13)$$

for zero-order kinetics where k_1 and k_0 are the first-order and zero-order rate constants at a specific temperature, respectively and D is the initial drug concentration. The predicted shelf lives are shown in

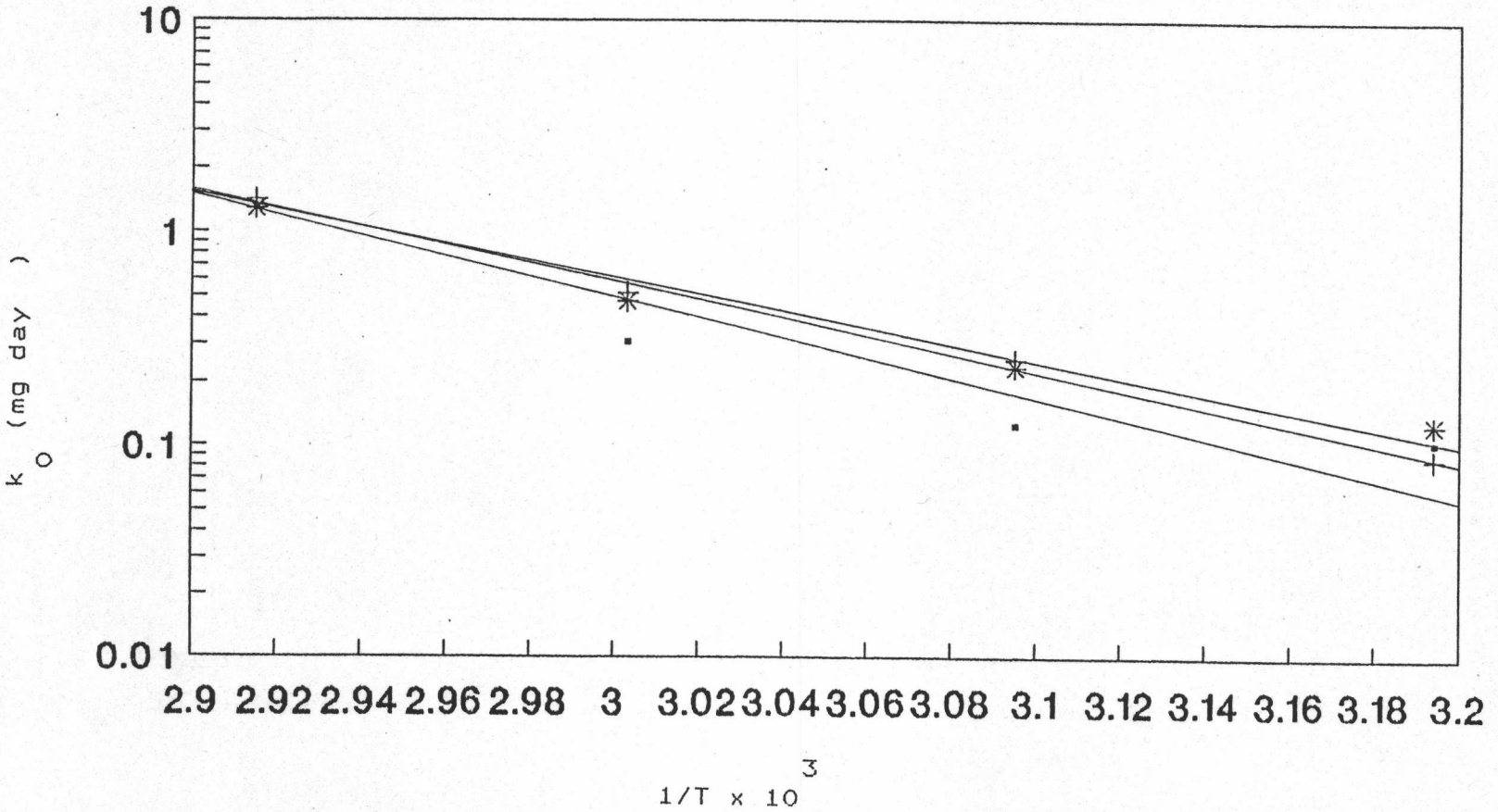

Figure 19. The Arrhenius plots of formulation No. 1, —•— ; formulation No. 3, —+— ; and formulation No.5, —*— ; obtained from zero-order kinetics.

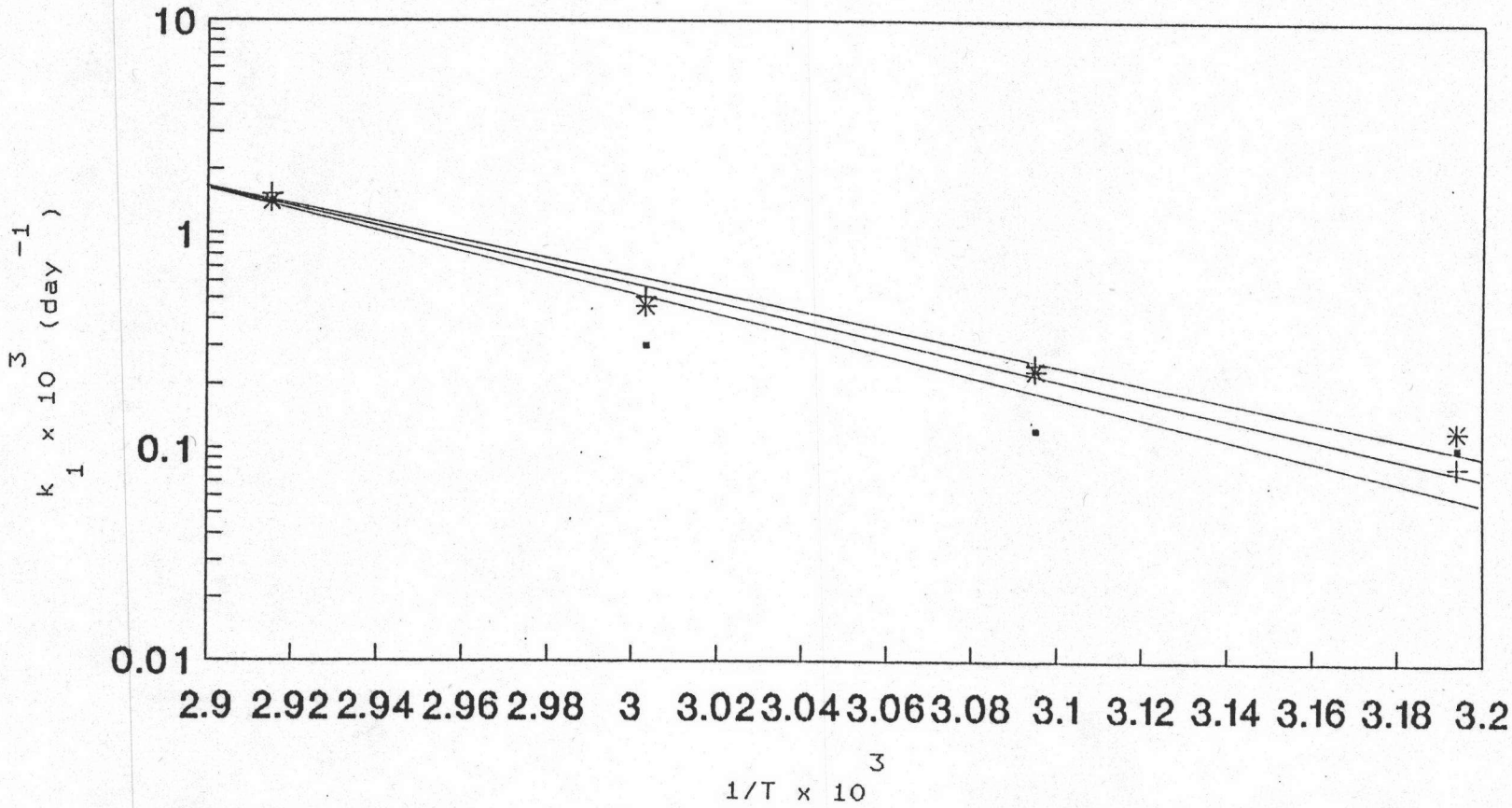

Figure 20. The Arrhenius plots of formulation No. 1, —•—; formulation No. 3, —|—; and formulation No.5, —×—; obtained from first-order kinetics.

Table 12 : The variables of Arrhenius equation of prepared indomethacin solutions

Formulation No.	zero-order		first-order	
	log A	Ea (k cal/mol)	log A	Ea (k cal/mol)
1	11.250	17.725 ± 4.605	8.931	18.745± 4.993
3	12.391	19.243 ± 1.031	10.129	20.360 ± 1.229
5	10.341	16.152 ± 1.664	7.990	17.131 ± 1.972

table 13. Note that the average laboratory room temperature was 33°C.

The estimates of predicted log k's from Arrhenius plot are variable and have error associated with them. The variance of a predicted value, Sp^2, is (Drapper and Smith, 1981):

$$Sp^2 = Sb^2 \left(1 + 1/N + (X_T - \bar{X})^2 / (X-\bar{X})^2\right) \qquad (16)$$

where N is the number of observations, X_T is the inversion of temperature of prediction and X is the average of the inversion of temperatures. The predicted value has a variance that consists of the error due to the estimation involved in the fitting plus the error associated with the new log k at the predicted temperature. A 95 % confidence interval of a predicted log k equals $t(2, 0.05)\ Sp^2$. Conservative expiration dates based on the time for 10 % decomposition are presented in table 13, according to the lower limit of the confidence interval. As can be seen that the predicted shelf lives estimated using the zero-order kinetics are less than those estimated using the first-ordedr kinetics. So, if a shelf life had to be predicted, the calculation using zero-order kinetics should be performed. From the data shown in table 13, the shelf lives of all three formulations are at least three years.

Table 13 : The predicted shelf lives ($t_{0.9}$) at room temperatures[a] of formulation No. 1, 3 and 5

Formulation No.	zero-order		first-order	
	predicted shelf lives (years)	conservative shelf lives (years)	predicted shelf lives (years)	conservative shelf lives (years)
1	7.01	4.52	8.23	7.62
3	6.16	5.43	7.43	7.28
5	4.29	3.48	5.06	4.90

[a] The average laboratory room temperature was 33 C.

Prepared Topical Indomethacin Solutions at Ambient Temperature

The stabilities of indomethacin solutions at ambient temperature (average of 33°C) had been studied for 230 days. The slopes of the concentration-time profiles (figure 21) and log (concentration)-time profiles (figure 22) are shown in table 14. Since the slopes are close to zero, their significance are tested using the t test with the null hypothesis Ho: B = 0 versus the alternative hypothesis Ha: B ≠ 0, where B is the slope values. The t statistics are obtained from

$$t\ (d.f., 0.975) = \frac{b}{Sb}$$

where b is a sample estimate of true slope, B, and Sb is its variance. The values of t statistics were refered to the t distribution with (N-2) degree of freedom at the significance level of 0.05. In this case the t statistics of formulation No. 3 (containing pluronic F127) is less than the t (26, 0.975) = 2.06. That is the null hypothesis Ho: B = 0 is accepted, i.e., the slope was zero. From the statistical standpoint, formulation No. 3 did not degrade chemically. The t statistics of formulation No. 1 (containing polysorbate 80) and formulation No. 5 (containing pluronic F68) were greater than the t (26, 0.975) = 2.06. Therefore the null hypothesis Ho: B = 0 were rejected. These were evidences that the slopes of

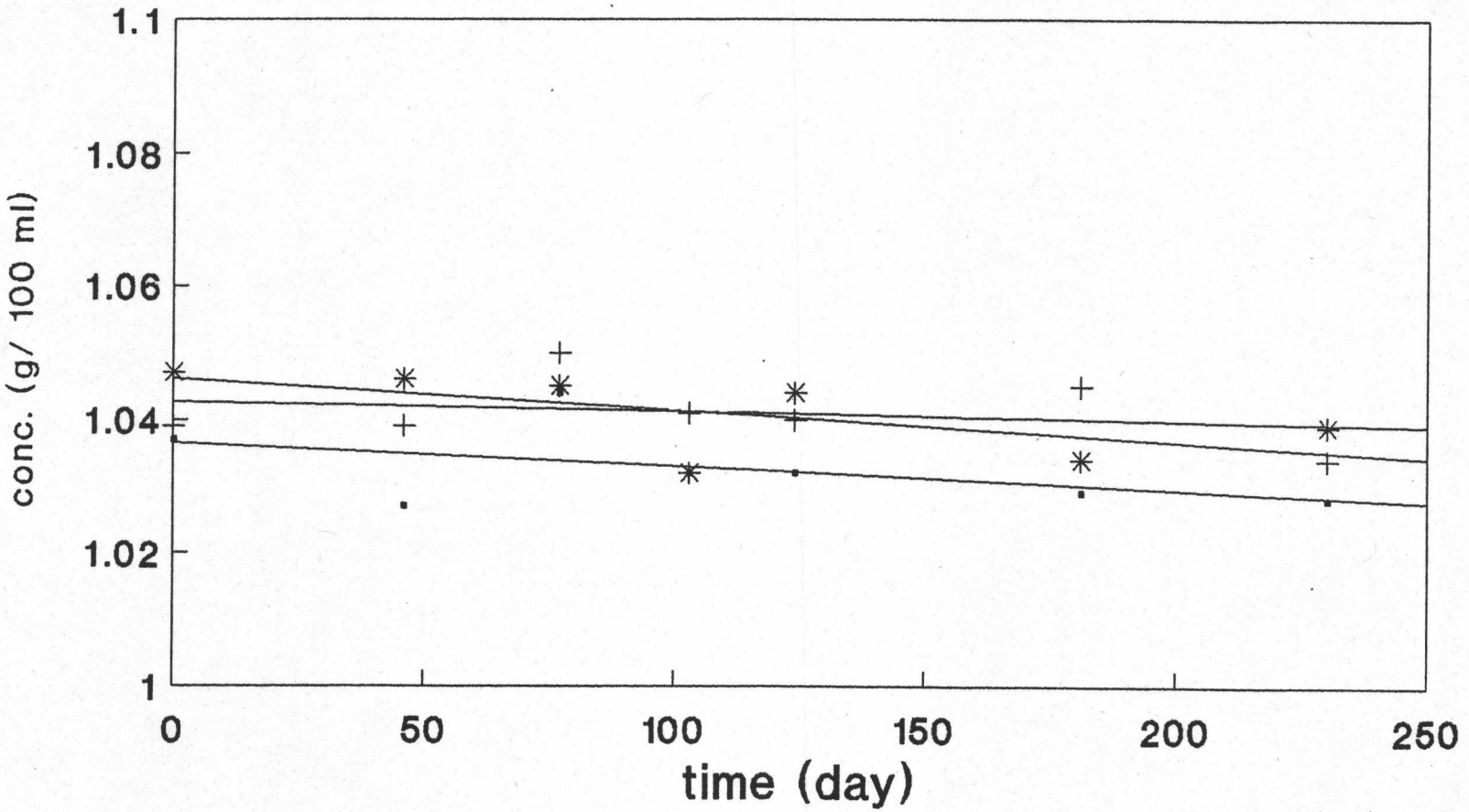

Figure 21. The concentration vs time plots of the prepared indomethacin solutions at ambient temperature (33°C) (Formulation No. 1, —•— ; Formulation No. 3, —+— ; Formulation No. 5, —*—).

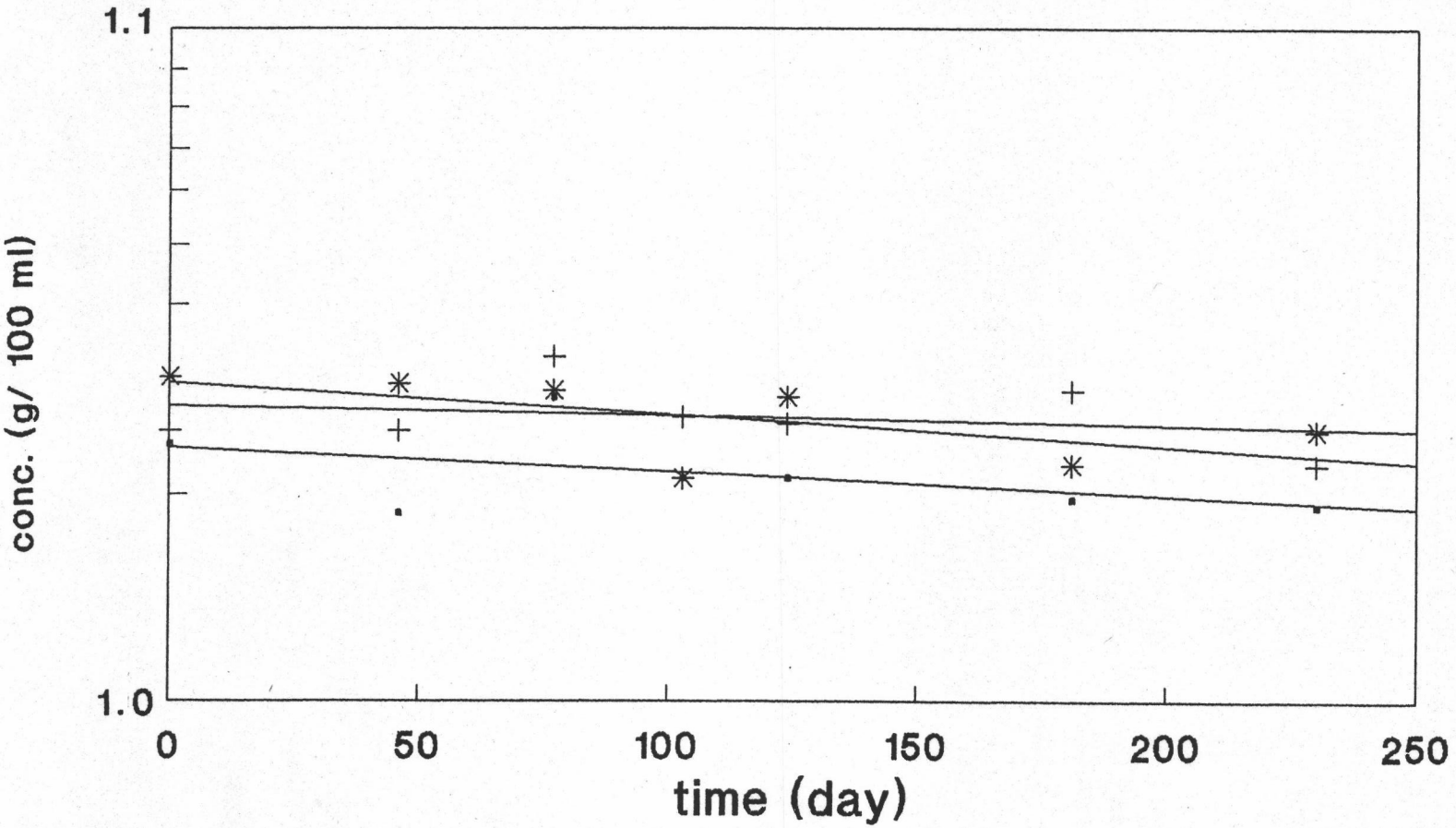

Figure 22. The semilog plots of concentration vs time of the prepared indomethacin solutions at ambient temperature (33°C) (Formulation No. 1, —•— ; Formulation No. 3, —+— ; Formulation No. 5, —✳—).

Table 14 : The rate constants and their t statistics of the prepared topical indomethacin solutions at ambient temperture

Formulation No.	zero-order		first-order	
	k_0 ($mg\ day^{-1}$)	t	$k_1 \times 10^3$ (day^{-1})	t
1	0.033± 0.015	2.11	0.031± 0.015	2.10
3	0.016± 0.022	0.92	0.015± 0.021	0.92
5	0.058± 0.017	3.37	0.056± 0.017	3.37

both formulation No. 1 and 5 are different from zero at the significance level of 0.05. In other words, formulation No. 1 and 5 had some degradations.

The rate constants of the prepared topical indomethacin solutions actually obtained at ambient temperature (average of 33°C) were compared to those estimated from the Arrhenius equation by the t test with the null hypothesis Ho: b = B versus the alternative hypothesis Ha: b ≠ B, where b is the actual rate constants obtained at ambient temperature and B is the predicted rate constants from the Arrhenius equation. The t statistics is obtained from

$$t\ (d.f., 0.975) = \frac{b - B}{Sb}$$

and is shown in table 15. The values of t statistics were refered to the t distribution with 26 degree of freedom which is 2.06 at a significance level of 0.05. All t statistics from the test are less than 2.06. Therefore the null hypothesis Ho: b = B were accepted. This could be concluded that the actual rate constants of the topical indomethacin solutions at ambient temperature are equal to the predicted rate constants obtained from the Arrhenius equation.

Stability Study of Elmetacin™

The rate constants obtained from the concentration-time profiles (figure 23) and the log

Table 15 : The comparison of rate constants actually obtained and the ones predicted

Formulation No.	zero order			first order		
	actual rate constants k_0 ($mg\ day^{-1}$)	predicted rate constants k_0 ($mg\ day^{-1}$)	t	actual rate constants $k_1 \times 10^3$ (day^{-1})	predicted rate constants $k_1 \times 10^3$ (day^{-1})	t
1	0.033 ± 0.015	0.039	0.40	0.031 ± 0.015	0.035	0.29
3	0.016 ± 0.022	0.044	1.27	0.015 ± 0.021	0.039	1.84
5	0.058 ± 0.017	0.064	0.35	0.056 ± 0.017	0.057	0.06

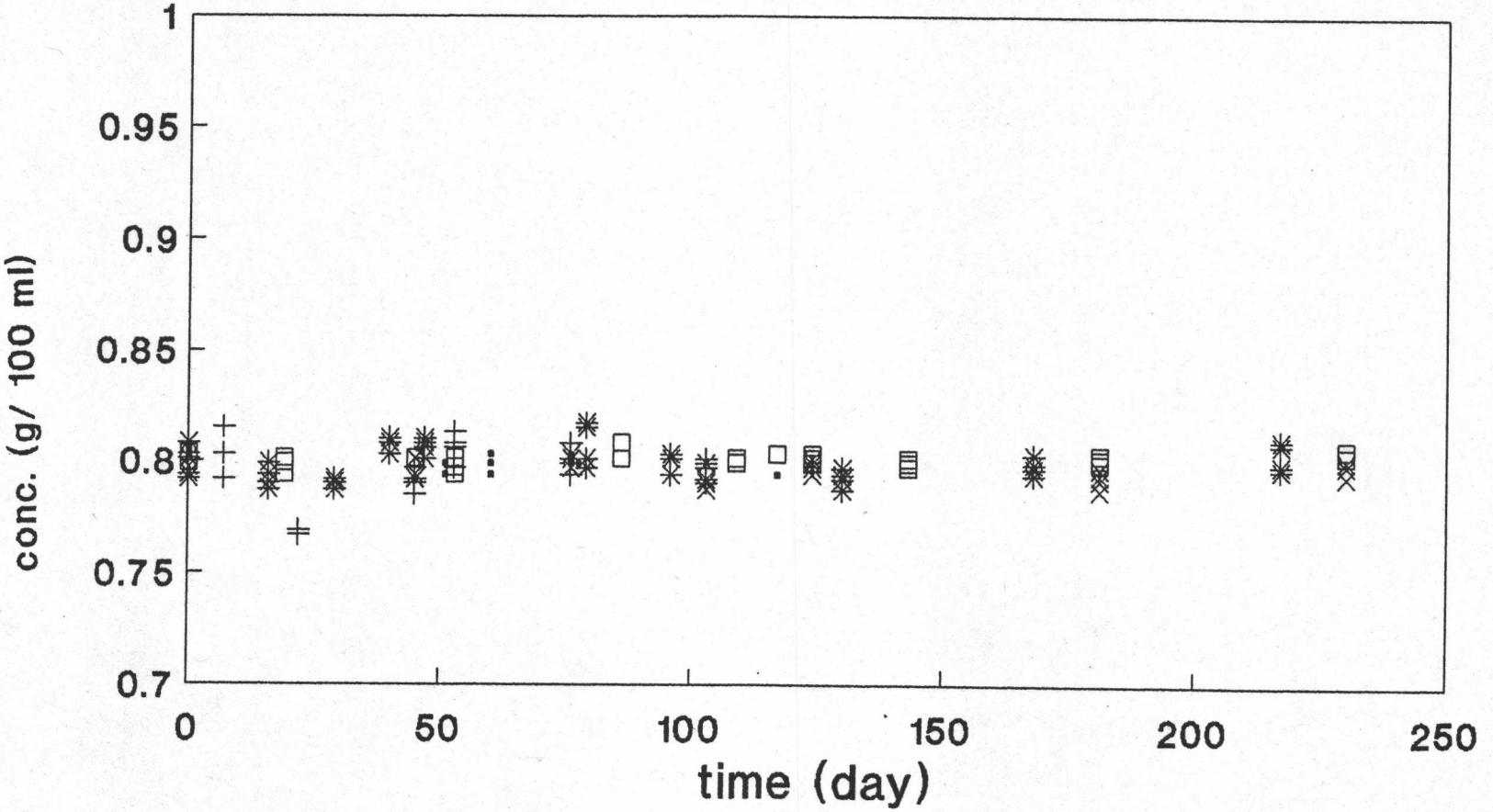

Figure 23. The concentration vs time plots of Elmetacin™ at elevated temperatures (70°C, —•— ; 60°C, —|— ; 50°C, —*— ; 40°C, —□— ; ambient temperature, —×—).

(concentration)-time profiles (figure 24) of Elmetacin™ at 40°C, 50°C, 60°C, 70°C and ambient temperature are reported in table 16. As can be seen that the rate constants or slopes is near zero, therefore, the t test for significance of the slopes (B) were performed with the null hypothesis Ho: B = 0 versus the alternative hypothesis Ha: B ≠ 0. All t statistics except that at 70°C are less than the reference values at the significance level of 0.05. Therefore, the null hypothesis is accepted, i.e., the rate constants at these temperatures are not different from zero. Only the rate constants of Elmetacin™ studied at 70°C are significant from the statistical standpoint. Consequently, the shelf life of Elmetacin™ could not be predicted using the Arrhenius equation. Therefore, it could be concluded that Elmetacin™ is very stable chemically and its chemical stability is higher than all prepared indomethacin solutions.

Physical Stability

Physical stabilities of all preparations had been observed all the time of study which were about 230 days. The prepared indomethacin solutions were pale yellow initially. Their odour had not changed but their colour were darken after storage especially at high temperature. Formulation No. 1 was darken finally at ambient temperature. The colour of formulation No. 3 and 5 had almost not changed over 230 days at ambient temperature.

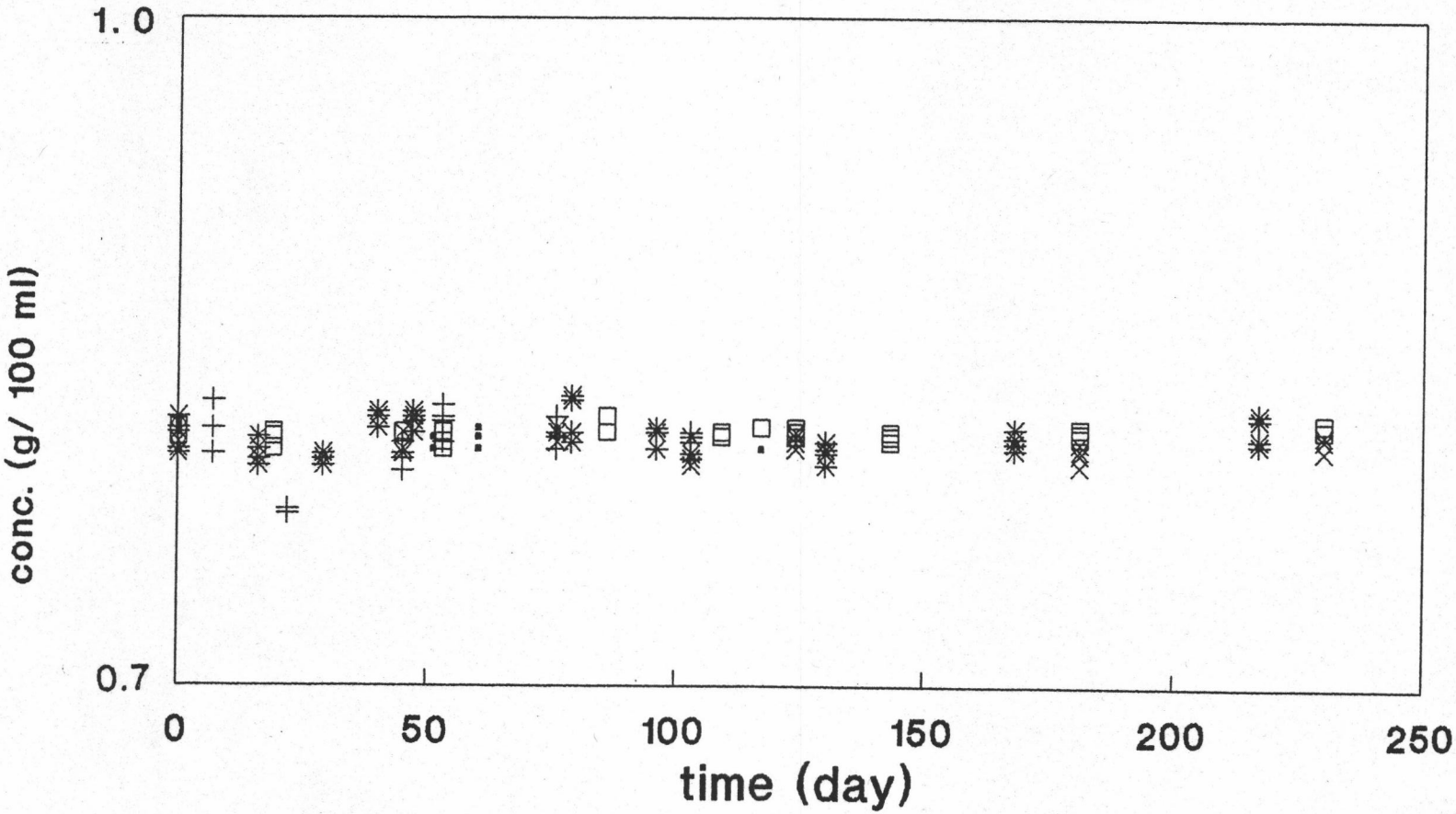

Figure 24. The semilog plots of concentration vs time of Elmetacin™ at elevated temperatures (70°C, —•— ; 60°C, —+— ; 50°C, —*— ; 40°C, —□— ; ambient temperature, —×—).

Table 16 : The rate constants and their t statistics of Elmetacin™ at various temperatures.

Temperature (°C)	zero order		first order		t (d.f., 0.975)
	k_0 ($mg\ day^{-1}$)	t	$k_1 \times 10^3$ (day^{-1})	t	
ambient temp	0.000 ± 0.012	0.00	0.000 ± 0.015	0.00	2.06
40	0.017 ± 0.006	-	0.021 ± 0.008	-	2.02
50	0.006 ± 0.019	0.35	0.008 ± 0.023	0.35	2.02
60	0.032 ± 0.072	0.94	0.087 ± 0.092	0.94	2.06
70	0.066 ± 0.017	3.97	0.082 ± 0.021	3.97	2.10

The reason for colour change might be that the drug was incompatible with other ingredients in the preparations such as propylene glycol. However, it was not relate to the chemical stability because the scan of the darken indomethacin solutions at the wavelength range of 200-500 nm were not different from that of the initial indomethacin solution. Elmetacin™ had no physical change at all.

Formulation of Gel

In general, the formula of a medicated gel preparation is composed of a drug or drugs, a gelling agent, a solvent or a levigating agent, a humectant, a preservative and/or other stabilizers and vehicles. In this formulation, it is necessary to have indomethacin in a dissolved form because the dissolved form of indomethacin was absorbed more effectively through the skin than the undissolved form (Naito and Tsai, 1981). The study of indomethacin solutions suggested that cosolvents and surfactants could be used to solubilize indomethacin. The cosolvents used in the study were alcohol, propylene glycol, water, and the surfactants used were pluronic F127, pluronic F68 and polysorbate 80. Fixing the ingredients used as in the case of topical solutions, topical gels were prepared by adding only a gelling agent. The gel preparations were performed as they were expected to be more stable than the solutions by the aid of the

increase in viscosity of formulation. The gel matrix might also prevent the attack of catalytic species. An appropriate gelling agent available for high alcoholic preparations was carbopol 940 (Rawlins, 1977). A preservative was not necessary in these preparations because the amount of alcohol were high enough to preserve the preparations.

The formulation of topical indomethacin gel (table 17) was based on the study of indomethacin solutions. The first formulation contained 20 % alcohol, 10 % propylene glycol and 20 % pluronic F127 and was called formulation No. 1. The reason for selecting pluronic F127 in this formulation was that pluronic F127 can form gel. However, pluronic F127 cannot form gel by itself in a hydroalcoholic preparation. It was expected that a preparation having both carbopol 940 and pluronic F127 can contain more alcohol than a preparation having only carbopol 940. In formulation No. 1, indomethacin was not dissolved as the gel was cloudy. Formulation No. 2 was therefore prepared by increasing alcoholic percentage to 30 % but indomethacin was still undissolved. Therefore, formulation No. 3 was performed by increasing alcohol concentration to 40 %. This formulation was clear but its appearance became viscous liquid. Therefore, the amount of pluronic F127 was varied and alcoholic percentage was also varied between 30 % and 40 %. The alcoholic concentration of 33 % was chosen because the gel could not be formed at the alcoholic percentages of more than 33 % and

Table 17 : The formulation of indomethacin gels

Formulation No.	1	2	3	4	5	6	7
Indomethacin (g)	1	1	1	1	1	1	1
Alcohol (ml)	20	30	40	33	33	33	30
Propylene glycol (g)	7-10	7-10	7-10	10	10	10	10
Pluronic F127 (g)	20	20	20	15-30	-	-	10
Pluronic F68 (g)	-	-	-	-	20-30	-	-
Polysorbate 80 (g)	-	-	-	-	-	12-30	12
Carbopol 940 (g)	1	1	1	1	1	1	1
Purified water to (ml)	100	100	100	100	100	100	100

All formulations were adjusted viscosity with 2% sodium hydroxide solution.

indomethacin could not be dissolved at those less than 33 %. The formulation No.4 gave clear gel. Polysorbate 80 and pluronic F68, respectively, were included instead of pluronic F127 in formulation No. 5 and 6 for comparison of their stabilities. The three surfactants used were varied in concentrations and the least concentrations that gave clear gels are 15 % pluronic F127, 20 % pluronic F68 and 12 % polysorbate 80 but clear crystals were formed after two months. The concentrations of the three surfactants were therefore increased up to 30 % in the formulation No. 4, 5 and 6. The only formulation that was absent of recrystallization is the one with 30 % polysorbate 80 but it was slimy when it was applied to the skin. This suggested that the combined surfactants should be used and 10 % pluronic F127 and 12 % polysorbate 80 were chosen as shown in formulation No. 7. This preparation gave rather physical-satisfying gel. This gel was clear and the crystals had not been formed during the time of study. Furthermore, the colour and odour of this gel had not changed for 3 months.

Stability Study of Gel

The rate constant obtained from the concentration-time profile (figure 25) and log (concentration)-time profile (figure 26) of this gel at ambient temperature were 0.619 ± 0.047 mg day^{-1} and $0.604 \pm 0.047 \times 10^{-3}$ day^{-1}, respectively. Since elevated temperatures affect gel

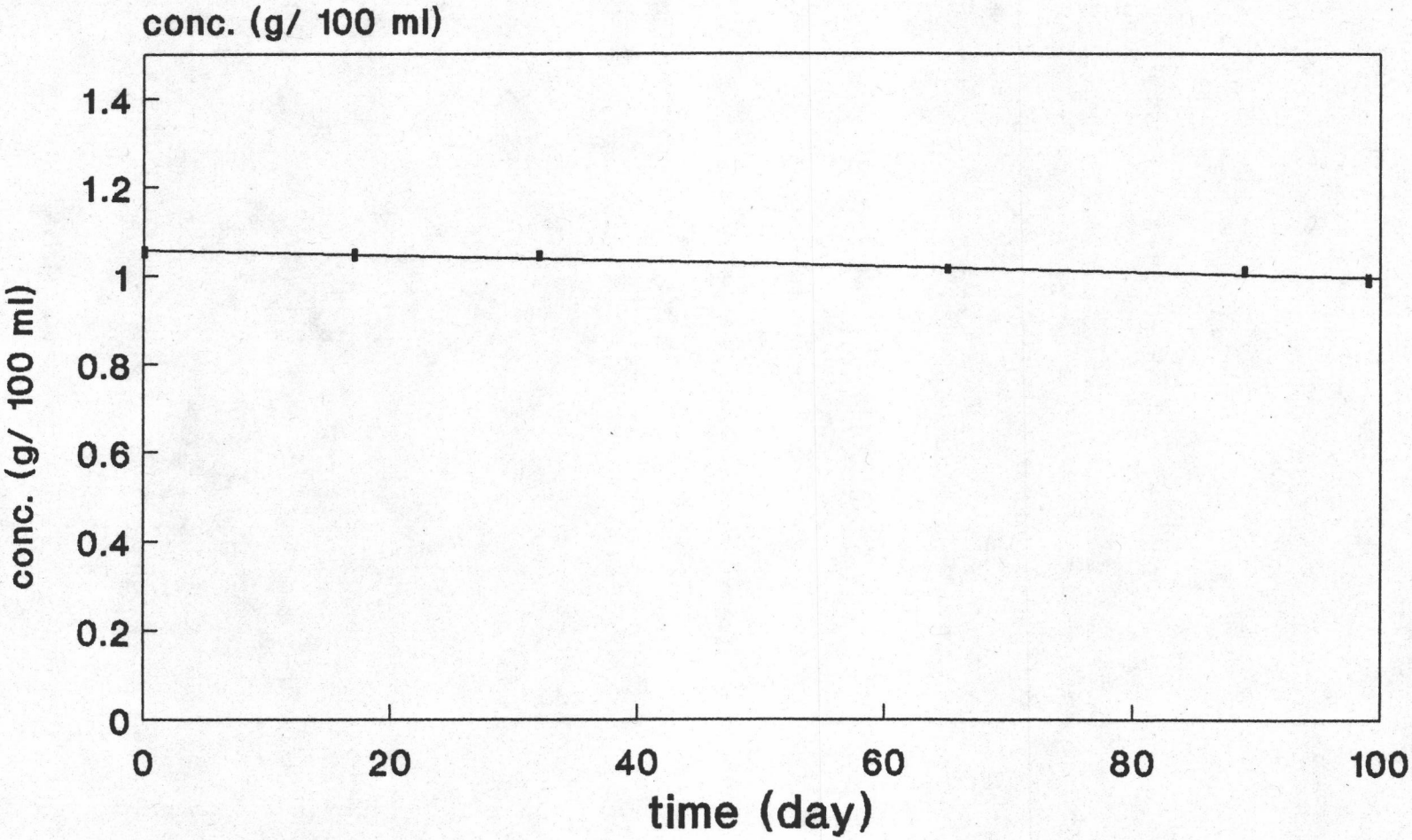

Figure 25. The concentration vs time plot of the prepared indomethacin gel at ambient temperature.

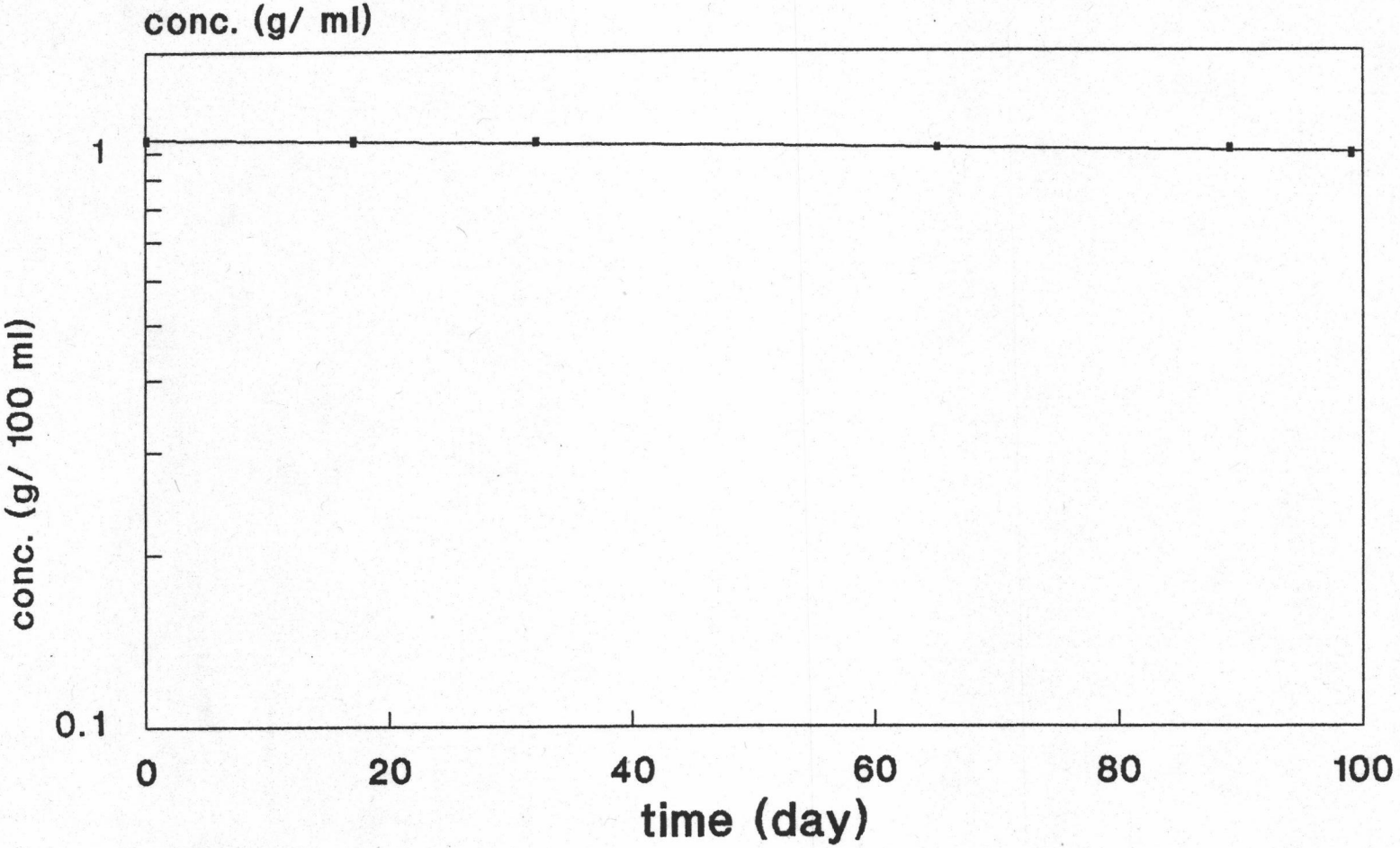

Figure 26. The semilog plot of concentration vs time of the prepared indomethacin gel at ambient temperature.

properties such as its viscosity. The predicted shelf lives were estimated from the rate constants at ambient temperature using equations 9 and 13 in Chapter II for first-order and zero-order calculations, respectively. The predicted shelf lives obtained from these calculations were 175 ± 14 days and 161 ± 10 days for first-order and zero-order kinetics, respectively. It can be seen that the prepared indomethacin gel was somewhat unstable.

It was expected earlier that the carbopol 940 would help preventing the degradation of indomethacin, i.e., the indomethacin gels would be more stable than the indomethacin solutions. However, the results showed that the indomethacin gels were less stable than the indomethacin solutions. A possible explanation is that the excess hydroxide ions used in the process of gel formation catalyzed the decomposition reaction of indomethacin. If a further study of indomethacin gel will be performed, other gelling agents which do not need a neutralizing base would be recommended as they could prevent the specific base catalysis.