CHAPTER IV

RESULTS AND DISCUSSION

1. Calibration curve determination.

1.1 The calibration curve data determined using a spectrophotometer expressed as the absorbance of piroxicam in 0.01 M methanolic hydrochloric acid were shown in Table 5 and Figure 10 , The regression equation obtained was "absorbance = 0.0013 + (0.0802 x conc.)". The coefficient of determination (r^2) was highly significant (0.9999). The coefficient of variation of the percent theories was calculated from the standard deviation over the mean and found to be less than 2 which could be accepted. This curve was used for calculating the solubility of piroxicam in cosolvents since the maximum absorbance at a wavelength of 334 nm was not altered by cosolvent at the dilution of measurement as seen in Figure 11.

1.2 The calibration curve data determined using HPLC expressed as the area ratio of piroxicam to tenoxicam (internal standard) were reported in Table 6 and Figure 12. The retention time of tenoxicam and piroxicam were 2.84 and 3.91 minutes, respectively ,as seen in Figure 13. The regression equation obtained was " area ratio = -0.0005 + (0.0483 x conc.) with coefficient of determination of

Table 5 : Calibration curve data of piroxicam in 0.01 M methanolic hydrochloric acid obtained from UV spectrophotometer

Std No.	Conc (ug/ml)	Absorbance	Inversely estimated conc[a]	% Theory[b]
1	1	0.094	1.0033	100.33
2	2	0.171	1.9636	98.18
3	4	0.333	3.9839	99.60
4	6	0.499	6.0542	100.90
5	8	0.657	8.0246	100.31
6	9	0.737	9.0223	100.25
7	10	0.814	9.9826	99.83
8	12	0.973	11.9655	99.71

Mean = 99.8888

S.D. = 0.8075

C.V[c] = 0.8084

a : Obtained from the fitted curve

absorbance = 0.0013 + (0.0802 x conc.); r^2 = 0.9999

$$\text{Inversely estimated concentration} = \frac{(\text{Absorbance} - 0.013)}{0.0802}$$

b : $$\% \text{ Theory} = \frac{\text{Inversely estimated concentration} \times 100}{\text{known conc}}$$

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

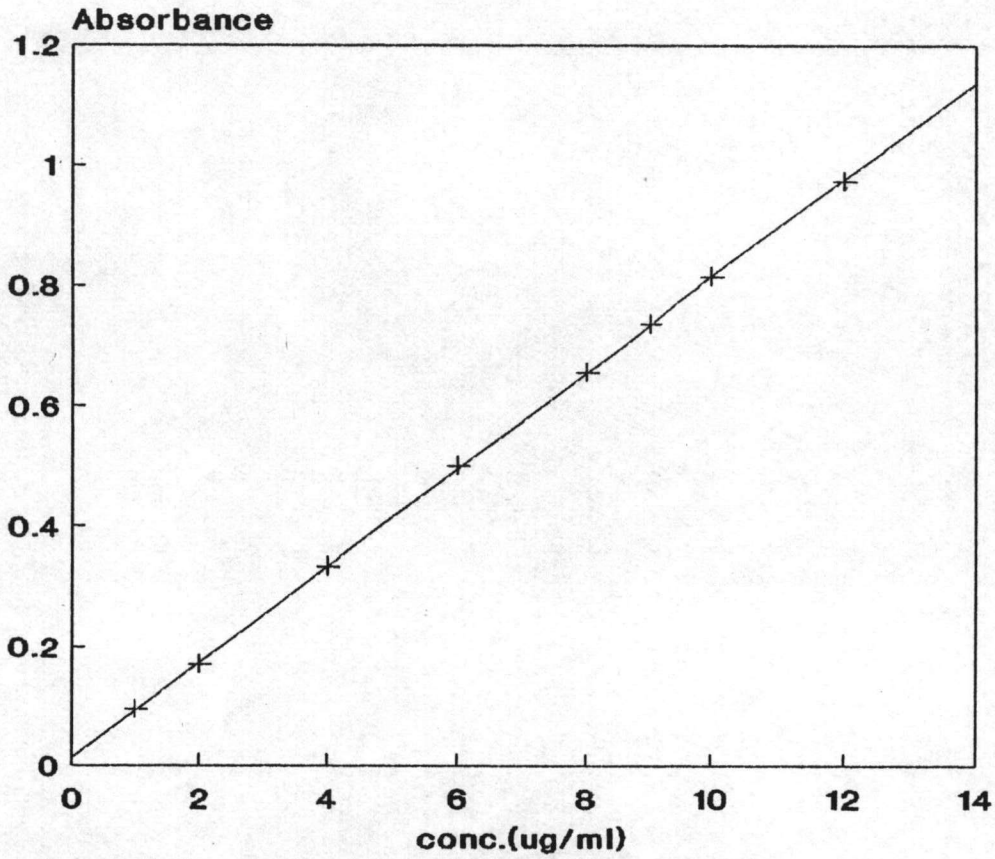

Figure 10 : Calibration curve of piroxicam from UV spectrophotometer

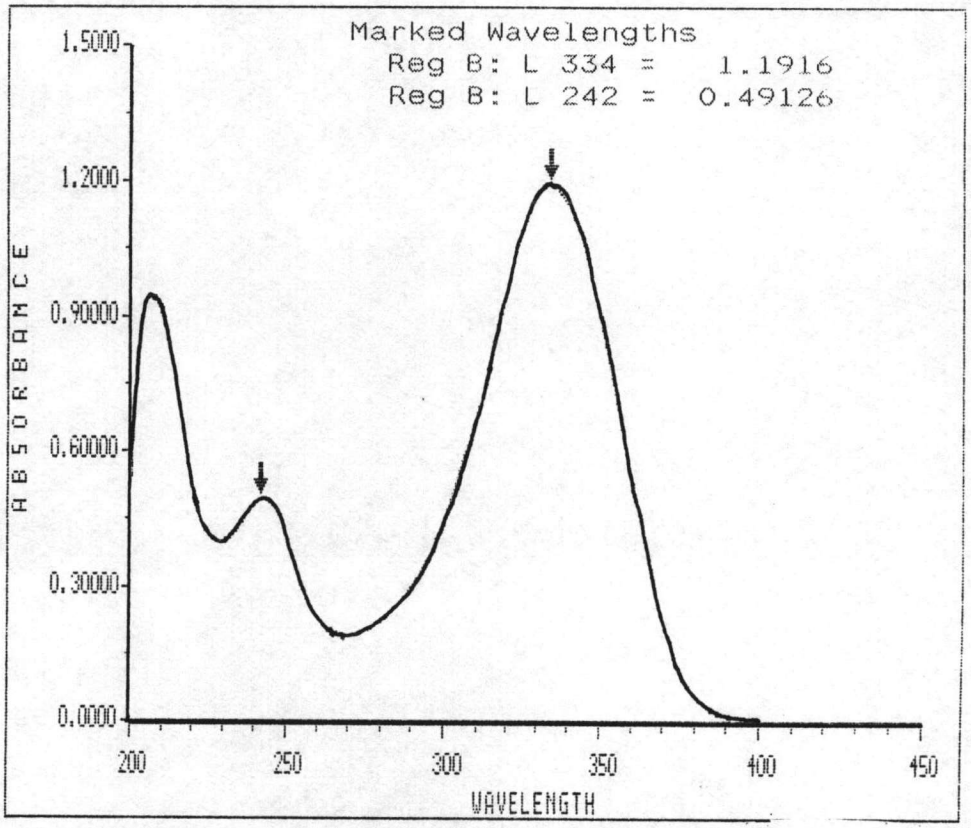

Figure 11 : UV spectrum curve of piroxicam in cosolvent in the dilution of measurement with 0.01 M methanolic hydrochloric acid

Table 6 : Calibration curve data of piroxicam in 0.01 M methanolic hydrochloric acid obtained from HPLC

Std No.	Conc (ug/ml)	Area ratio[a]	Inversely estimated conc.[b]	% Theory[c]
1	10.4	0.50085	10.377	99.78
2	20.8	0.99025	20.509	98.60
3	31.2	1.49420	30.942	99.17
4	52.0	2.50410	51.850	99.71
5	72.8	3.60590	74.660	102.55
6	93.6	4.49690	93.106	99.47
7	104.0	4.99200	103.356	99.38

Mean = 99.809

S.D. = $\pm$ 1.271

C.V[d] = 1.2734

a : Area ratio = $\frac{\text{area under the curve of sample}}{\text{area under the curve of internal std.}}$

b : Obtained from the fitted curve

area ratio = -0.0005 + (0.0483 x conc), r^2 = 0.999

Inversely estimated concentration = $\frac{(\text{Absorbance} + 0.0005)}{0.0483}$

c : % Theory = $\frac{\text{Inversely estimated concentration x 100}}{\text{know concentration}}$

d : % Coefficient of variation = $\frac{\text{S.D. x 100}}{\text{Mean}}$

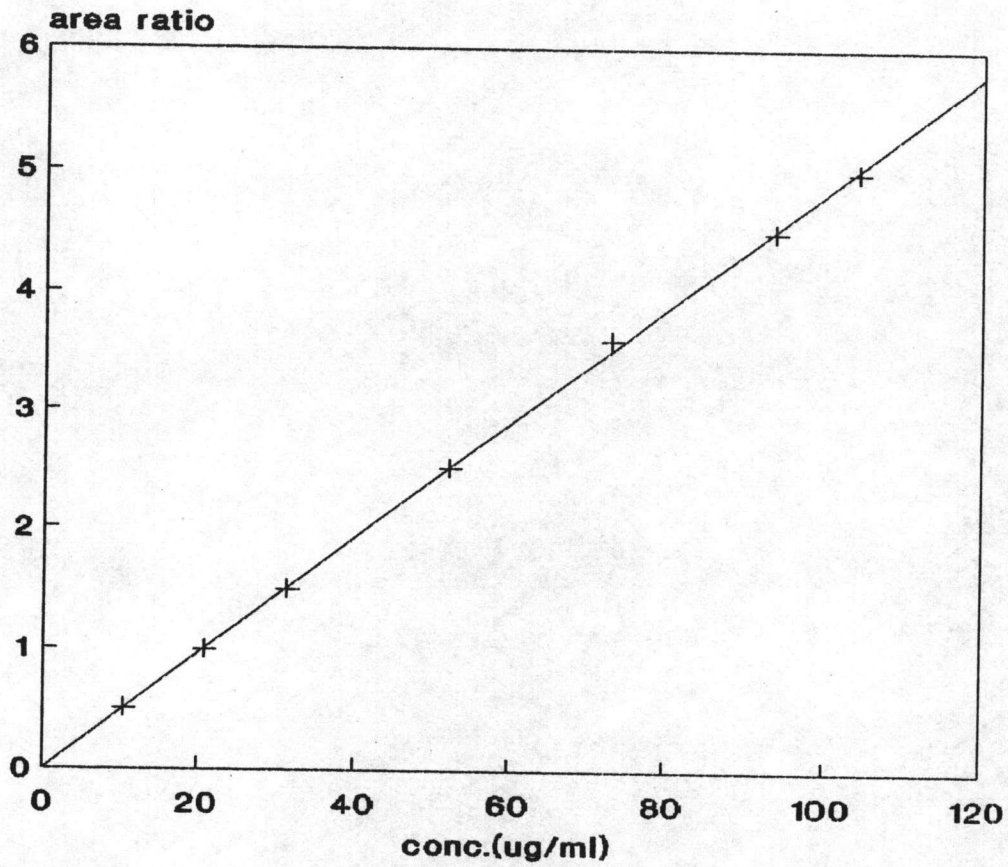

Figure 12 : A calibration curve of piroxicam (HPLC)

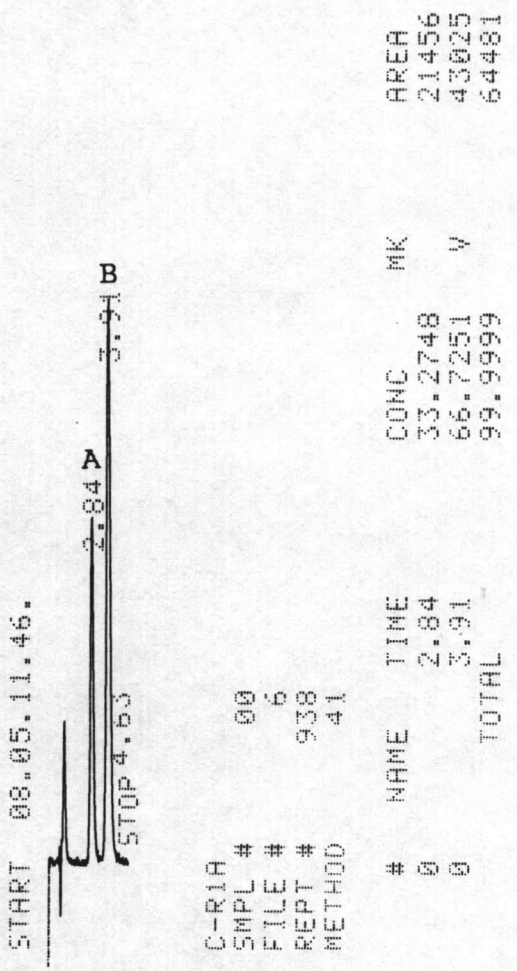

Figure 13 : A chromatogram of piroxicam and (internal standard) tenoxicam.

Key : A = tenoxicam retension time 2.84 mins

B = piroxicam retension time 3.91 mins

0.9999. Again the coefficient of variation of the percent theories was less than 2 which was acceptable. The calibration curve of this method was used for calculating the concentration of piroxicam solution when there was nicotinamide as complexing agent instead of UV spectrophotometric method This was because the maximum absorption of UV spectrophotometry was altered by nicotinomide at the studied concentration but HPLC method wasn't. So the HPLC method was more suitable for piroxicam with nicotinamide solution.

2. Solubility of Piroxicam

The study of solubility of piroxicam using cosolvent and complexing agent was conducted in order to increase the solubility of piroxicam, as well as to search for the most appropiate cosolvent and amount of complexing agent to be added in the formulation process. The results showed that both methods could increase the solubility of piroxicam as expected. All results were detailed as follows.

2.1 Solubility of piroxicam using cosolvents.

Figure 14 illustrated the solubility data obtained for piroxicam in cosolvents of N, N dimethyl-acetamide, dimethylformamide, propyleneglycol, polyethylene glycol 400 and ethyl lactate with varying concentrations in water. These data indicated that logarithmic increase in

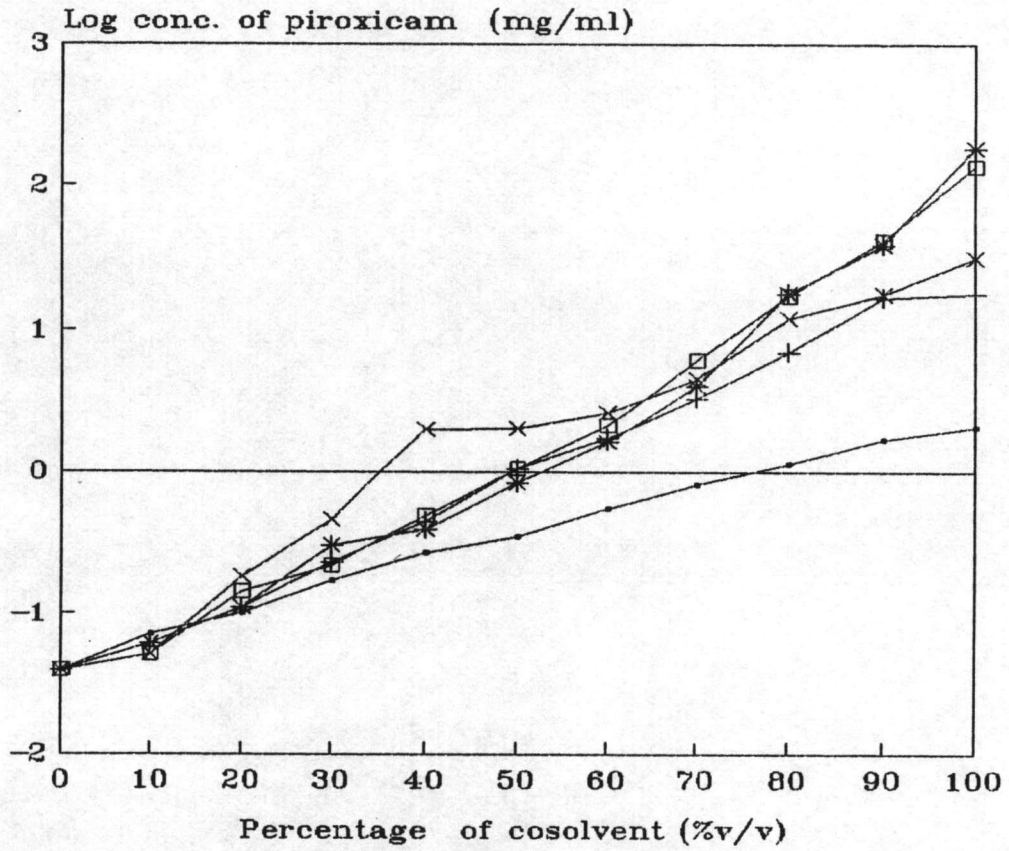

Figure 14 : Solubility of piroxicam in cosolvent

Key : —+— Propylene glycol

—•— Polyethyene glycol 400

—⊡— N,N,dimethylacetamide

—*— Dimethylformamide

—×— Ethyllactate

solubility occurred as the volume fraction of non aqueous solvents in the mixture was increased. The solubilities data expressed as mg/ml of piroxicam were presented in Tables 7-11. The data indicated that only dimethyl formamide (90.0% v/v) and N, N dimethylacetamide (90.0% v/v) could produce the solubility of the drug greater than 20 mg/ml. However ,the volume fraction of non-aqueous solvent was so high that might cause toxicity. A 50% v/v solution of dimethylacetamide had been reported to produce toxicity (Spiegel and Noseworthy,1963). Other solvents, polyethylene glycol 400, propylene glycol and ethyl lactate could improve the solubility of piroxicam, but results were less than the desired solubility. So other methods were considered to achieve the solubility target in combination with cosolvent. The process was started by selecting the type of cosolvent with limiting its ratio (% v/v), its safety (D_{50}) and low cost. Propylene glycol was chosen because of these specifications (hightest LD_{50} value, and low cost) (Spiegel and Noseworthy, 1963). The ratio of propylene glycol used was limited to 20-60% v/v). Other method was then introduce into cosolvent system.

2.2 Solubility of piroxicam using complexing agent.

The cosolvent technique alone could not increase the solubility of piroxicam to the desired level (at least 20 mg/ml). Thus, another method must be applied by using nicotinamide as a complexing agent.

Table 7 : Solubility of Piroxicam in cosolvents of polyethylene glycol 400 and water

Cosolvent No.	Polyethylene glycol 400 (% v/v)	Solubility of Piroxicam (mg/ml)
1	10	0.07
2	20	0.10
3	30	0.17
4	40	0.27
5	50	0.35
6	60	0.55
7	70	0.80
8	80	1.11
9	90	1.69
10	100	2.11

Table 8 : Solubility of piroxicam in cosolvents of propylene glycol and water

Cosolvent No.	Propylene glycol (% v/v)	Solubility of Piroxicam (mg/ml)
1	10	0.06
2	20	0.11
3	30	0.23
4	40	0.45
5	50	1.05
6	60	1.74
7	70	3.29
8	80	7.16
9	90	16.41
10	100	17.76

Table 9 : Solubility of piroxicam in cosolvents of dimethyl formamide and water

Cosolvent No.	Dimethyl formamide (% v/v)	Solubility of Piroxicam (mg/ml)
1	10	0.06
2	20	0.11
3	30	0.30
4	40	0.39
5	50	0.84
6	60	1.64
7	70	4.11
8	80	17.90
9	90	38.01
10	100	180.99

Table 10 : Solubility of piroxicam in cosolvents of N, N dimethylacetamide and water

Cosolvent No.	N, N dimethylacetamide (% v/v)	Solubility of Piroxicam (mg/ml)
1	10	0.05
2	20	0.14
3	30	0.22
4	40	0.49
5	50	1.04
6	60	2.13
7	70	6.15
8	80	16.88
9	90	40.62
10	100	135.98

Table 11 : Solubility of piroxicam in cosolvents of ethyl lactate and water

Cosolvent No.	Ethyl lactate (% v/v)	Solubility of Piroxicam (mg/ml)
1	10	0.05
2	20	0.18
3	30	0.46
4	40	1.98
5	50	2.03
6	60	2.62
7	70	4.56
8	80	12.13
9	90	17.53
10	100	30.77

Figure 15 illustrated the solubility of piroxicam in various concentratisons of nicotinamide. The phase solubility diagram of piroxicam showed positive curvatures similar to those observed with nicotinamide (Harte and Chen, 1979; Truelove et al, 1984; Hamza and Paruta, 1985; Rasool et al, 1991). It was probable that the enhancement of the aqueous solubility of piroxicam was due to complex formation of nicotinamide and piroxicam. This was seen by the phase solubility diagram of type A which indicated the formation of soluble complexs (Yalkowsky , 1981). The concentration range of nicotinamide in this study was from 0-450.00 mg/ml , providing the solubility of piroxicam to be ranged from 0.18-1.99 mg/ml (Table 12). The concentration of nicotinamide used in the formula should not be exceeded the concentration intake per day.

Nicotinamide was usually used in the treatment and prevention of nicotinic acid deficiency. Dose of up to 500 mg daily had been recommended. (Reynolds, 1989) It appeared that nicotinamide complexation was a useful approach to the enhancement of the solubility of piroxicam, although lower than the target level for formulating the piroxicam injection and the nature of the complex formed was not clear.

2.3 Solubility of piroxicam in cosolvent and complexing agent.

From sections 2.1 and 2.2 as discussed

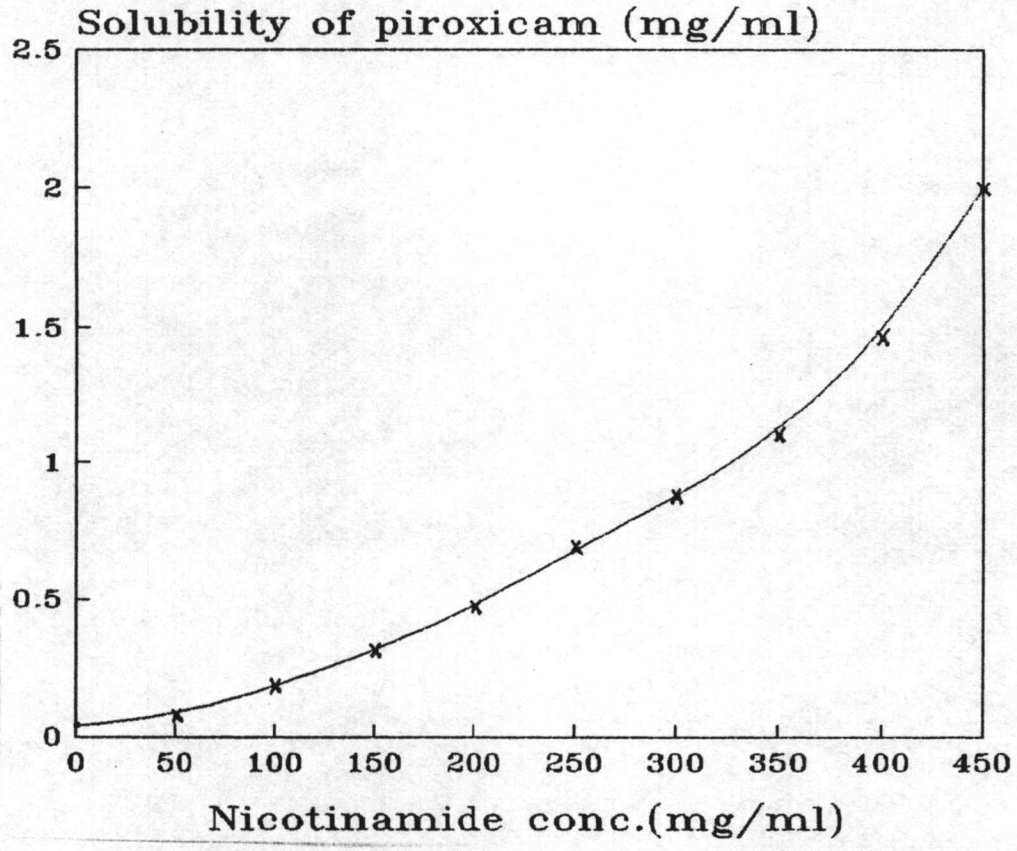

Figure 15 : Solubility of piroxicam in nicotinamide solution

Table 12 : Solubility of piroxicam in nicotinamide solution

Tube No.	Nicotinamide conc. (mg/ml)	Solubility of Piroxicam (mg/ml)
1	0	0.07
2	50	0.18
3	100	0.31
4	150	0.47
5	200	0.68
6	250	0.87
7	300	1.10
8	350	1.46
9	450	1.99

earlier, neither cosolvents nor nicotinamide could adequately provide the target solubility. Also, from the study of Truelove et al (1984) showed that cosolvent could increase solubility of nicotinamide-drug-complex. Hence, it was necessary that propylene glycol water cosolvent system and nicotinamide together were chosen to increase the solubility of piroxicam, with limiting concentration of nicotinamide from 150.00-250.00 mg/ml and concentration of propylene glycol in water from 20-60% v/v.

Figure 16 showed the phase solubilities diagram of piroxicam after using nicotinamide and cosolvent mixture of propylene glycol and water. The phase solubility diagram showed that the increase of solubility of piroxicam appeared to be related with both increase of nicotinamide concentration and propylene glycol's ratios. When the ratio of propylene glycol increased from 20-40% v/v the solubility of piroxicam could be increased at any concentration of nicotinamide. But when the ratio exceeded 40% v/v the solubilities of piroxicam did not increase. Data from Table 13 showed that propylene glycol enhanced the solubilities of piroxicam nicotinamide complex by more than 2 fold as expressed in mg/ml. This might be due to the effect of cosolvent (propylene glycol) in adjusting an appropiate environment for piroxicam nicotinamide complex formation. The addition of a cosolvent could reduce polarity or dielectric constant of water or drug solution (Yalkowsky and Roseman, 1981). But the mechanism of this event was not clear and needed further studies.

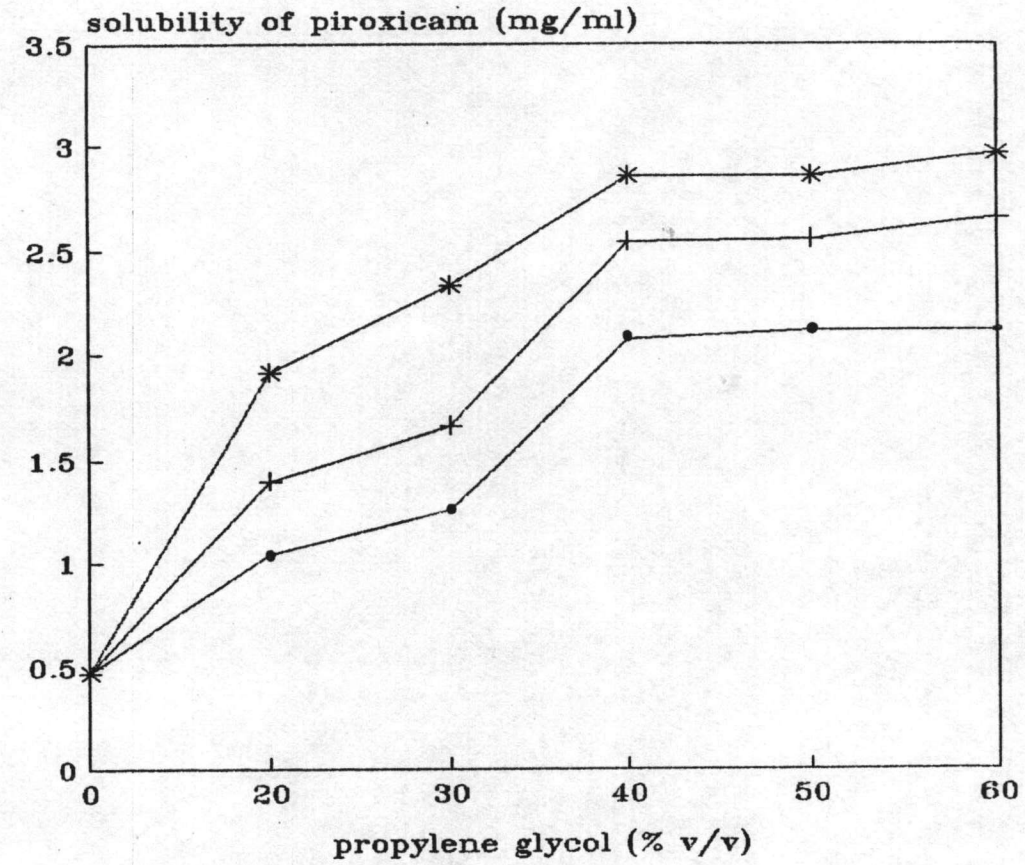

Figure 16 : Solubility of piroxicam in nicotinamide & propylene glycol.

Key : nicotinamide conc. (mg/ml)

150 200 250

Table 13 : Solubility of piroxicam in nicotinamide and propylene glycol solution

Tube No.	Propylene glycol % (v/v)	Solubility of piroxicam (mg/ml)		
		Concentration of nicotinamide in propylene glycol (mg/ml)		
		(150)	(200)	(250)
1	20	1.04	1.40	1.91
2	30	1.26	1.46	2.33
3	40	2.07	2.54	2.85
4	50	2.11	2.55	2.85
5	60	2.11	2.65	2.96

Thus from the study, the appropriate ratio of cosolvent for increasing the solubilities of piroxicam should be at 40% v/v, and the concentration of nicotinamide in propylene glycol should be 250 mg/ml. This combination could give reasonably solubilities of piroxicam. However, even if using cosolvent adjuncted with nicotinamide complexing agent, the desired solubility was inadequate. So other mean for improving the solubility of piroxicam was employed. This was accomplished by adjusting the pH of the solution using buffer systems.

2.4 Solubility of piroxicam in cosolvents and complexing agents at various pH of buffer systems.

From the work of Tsai, Hsu and Naito(1984); the solubilities of piroxicam in phosphate buffer pHs 2-10 showed that as the pH was increased the solubilities of piroxicam was increased as well. In order to achieve the desired solubility, the use of buffer to adjust pH was an alternative approach in this experiment, besides cosolvency and complexation. The buffer systems used in this study were phosphate buffer and Mc. Ilvaine (citrate phosphate buffer) which were the biological buffers. Fixing of propylene glycol at 40% v/v and nicotinamide concentration at 250mg/ml the buffer system were then used for adjusting pH from 5-8 which were appropiate pHs for intramuscular injections (Avis et al, 1984). Results in Table 14 and Figure 17 showed that above pH 7 of Mc Ilvaine buffer system, the solubilities of piroxicam exceeded 20 mg/ml. In constrast to the phosphate

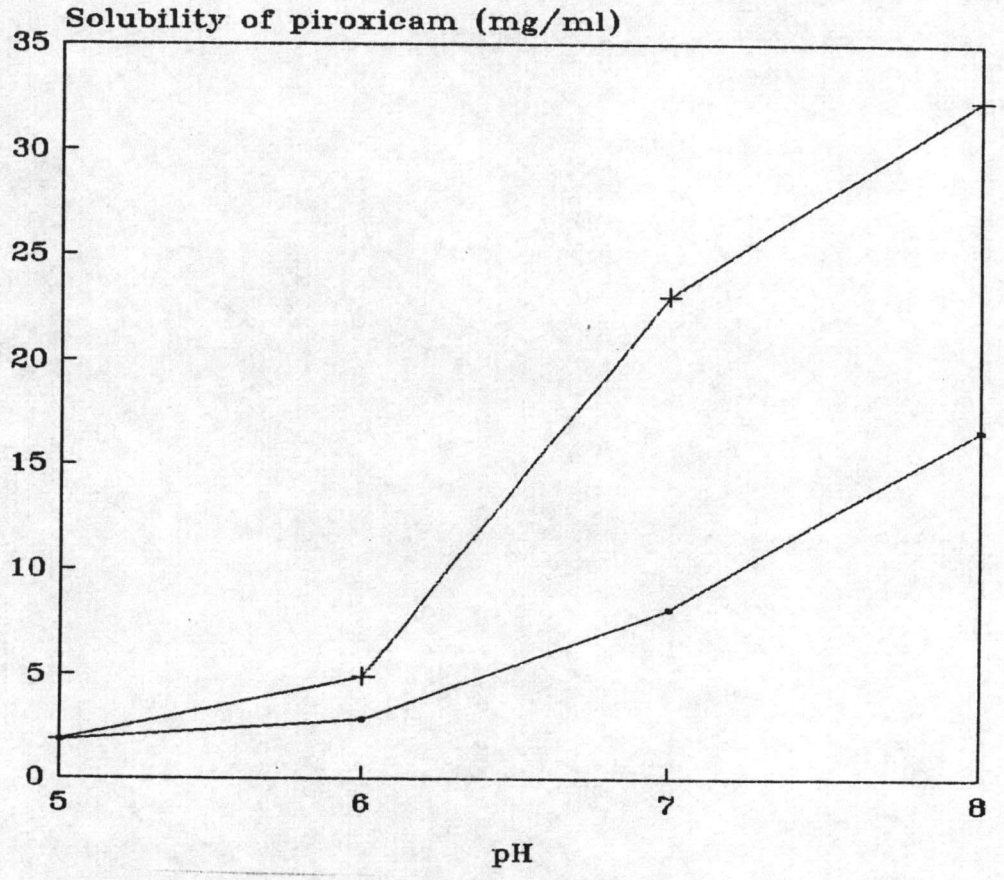

Figure 17 : Solubility of piroxicam in 250 mg nicotinamide, 40% propylene glycol, buffer solution.

Key : —•— phosphate buffer

—|— Mc.Ilvaine buffer

Table 14 : Solubility of piroxicam in 250 mg nicotinamide in propylene glycol (40% v/v in water) and buffer solution

pH	Solubility of Piroxicam (mg/ml)	
	Phosphate buffer	Mc. Ilvaine
5	1.84	1.88
6	2.83	4.89
7	8.11	22.98
8	16.65	32.29

buffer, even if pH rose to 8, the solubilities of piroxicam still not reached the target concentrations. It was evident from the data that solubility of piroxicam in each buffer system was significantly enhanced by increasing the pH. However, the solubility was increased in a non linear fashion as a function of increasing pH.

3. Formulation

3.1 Formulation of piroxicam injection

The piroxicam injections were formulated using a typical formula as follow:

Rx.

Piroxicam	20 mg.
Propylene glycol	0.4 ml.
Nicotinamide	250 mg.
Additive qs.	
Water for injection qs	1 ml.

According to the solubility study, the target solubility was reached after using nicotinamide at the concentration of 250 mg/ml,in combination with 40% v/v propylene glycol, and Mc. Ilvain (citrate phosphate buffer) starting from pH7 to higher. Therefore in order to formulate piroxicam injection, solution of pH 7 and pH 7.5 were chosen since at these pHs piroxicam was stable (Fini and Rabasco, 1992), and they were nearly the ideal pH for injection (pH of blood was 7.4, extreme deviation from this pH can cause complication)(Avis, Lachman and Lieberman,1984). Even if the solubility of piroxicam was greater and more stable above pH 7.5 (Fini and Rabasco, 1992). Those pHs were excluded because nicotinamide was incompatible with alkaline solution (Mc Evoy, 1989).

In general formulation method, some added

substances such as antioxidants, antimicrobial etc. frequently were incorporated into parenteral formular to provide stable, efficacious and elegant parenteral dosage form (Avis, Lachman and Lieberman, 1984;Lachman,Lieberman and Kanig,1986). The main purpose for the use of antioxidants in the preparation was to retard or prevent the oxidative breakdown of active ingredients upon their exposure to atmosphere oxygen. In this experiment, sodium sulfite was used as an antioxidant for the purpose of stabilizing agent at the concentration of 0.15% which was the normal concentration of sodium sulfite used in the preparation. Other reasons were that it was water soluble, more stable and effective agent as an antioxidant at a solution of pH 7 to 10 (Akers, 1982).

Benzyl alcohol at a concentration of 2%, usual concentration in parenteral products, (Avis, Lachman and Lieberman, 1984; Lachman,Lieberman and Kanig,1986.) was also included in the formulas. Besides the antimicrobial action, benzyl alcohol acted as an anesthetic in intramuscular injection (Reynolds, 1989).

3.2 Analysis of active ingredient in the formulation.

The percent labeled amount of formulated piroxicam injection was not less than 90%, and not more than 110% of the label claimed.Meanwhile that of the innovator's product was about 100-101% (Table 15). Thus the amount of active ingredient (piroxicam) in all formulas used

Table 15 : Percent lebeled amount of the formulas

Fommula No	Run 1		Run 2		Run 3	
	Conc[1]	% LA[2]	Conc[1]	% LA[2]	Conc[1]	% LA[2]
1	19.3685	96.84	19.9133	99.57	19.9932	99.97
2	18.7201	93.60	18.9464	94.73	19.1308	95.65
3	19.2573	96.29	19.0443	95.22	19.5815	97.91
4	19.2421	96.21	19.3039	96.52	19.1394	95.70
5	19.3371	96.69	19.0548	95.27	19.6414	98.21
6	19.0053	95.03	19.2449	96.22	19.7612	98.81
7	19.3457	96.73	19.6043	98.02	19.9513	99.76
8	19.4617	97.31	19.7555	98.78	19.1536	95.77
Innovator's	20.0538	100.27	20.3685	101.84	20.2221	101.11

Conc[1]. = Concentration in mg per ml

% LA[2] = Percent labeled amount of the drug

in stability study were within the limit as specified by the government regulation.

4. Stability study.

The stability of formulated piroxicam injections and an innovator's product were evaluated using serveral parameters. These parameters included amount of remained active ingredient, pH, crystal formation and color.

4.1 Observation on physical changes.

The physical changes in this experiment included pH, crystal formation and color.

4.1.1 pH

The pHs of all formulations were measured using pH metre. The results were shown in Table 16. As could be seen, there were no significant changes of pH in all formulations. This implied that the buffer capacity was adequate for maintaining the pH of the product at a constant value during the time of study.

4.1.2 Crystal formation

All formulation of piroxicam injections stored at normal room temperature (about 32°C) did not exhibit any changes in crystal formation (Table 17). In

Table 16 : pH value at mormal room temperature

Formula	Day								
	7	14	21	28	35	42	49	56	63
1	7.5	7.5	7.5	7.5	7.5	7.5	7.6	7.5	7.5
2	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
3	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
4	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
5	7.0	7.0	7.0	7.0	7.0	7.1	7.1	7.2	7.1
6	7.0	7.0	7.0	7.0	7.0	7.0	7.1	7.1	7.1
7	7.0	7.0	7.0	7.0	7.1	7.1	7.1	7.1	7.1
8	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.1	7.1
Innovator's product	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0

Table 17 : Crystal formation at normal room temperature

Formula	Day								
	7	14	21	28	35	42	49	56	63
1	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-
Innovator's product	-	-	-	-	-	-	-	-	-

- = negative crystal

contrast, those stored in refrigerator (temperature about 8 °C), as seen in Table 18, showed that crystal formations were observed starting from day 21 (formula 5), day 35 (formula 7) day 42 (formulas 6 and 8) and day 63 (all formulas except an innovator's product). This might be because (1) as the temperature decreasedr, the aqueous solubilities of any substances might be less. (2) in reversible reaction of the complex, a few amount of piroxicam was formed from reversed reaction and crystallized. (3) an excessive amount of nicotinamide in complex solution could crystallize too.

Another factor for the crystallization should be pH which could affect drug solubility. As could be seen that formulas with higher pHs (formulas 1, 2, 3 and 4) slowed crystal formation later than those with lower pHs (formulas 5, 6, 7 and 8). This might be dued to the solubilities of piroxicam-nicotinamide complex was affected from pH. These data (Table 14) were correlated with those of Tsai, Hsu, and Naito (1984) which established a positive relationship between piroxicam solubility and pH. However, the innovator's formula with pH about 8, did not exhibit any crystallization during the time observed at any temperature. Thus, it could be suggested that (1) all formulated formulas should not be kept in refrigerated temperature (2) formulation with higher pH could solve crystal formation problem.

Table 18 : Crystal formation at refrigerated temperature

Formula	Day								
	7	14	21	28	35	42	49	56	63
1	-	-	-	-	-	-	-	+	+
2	-	-	-	-	-	-	-	-	+
3	-	-	-	-	-	-	-	+	+
4	-	-	-	-	-	-	-	-	+
5	-	-	+	+	+	+	+	+	+
6	-	-	-	-	-	+	+	+	+
7	-	-	-	-	+	+	+	+	+
8	-	-	-	-	-	+	+	+	+
Innovator's product	-	-	-	-	-	-	-	-	-

- = negative crystal

+ = positive crystal

4.1.3 Color

All formulated injections and an innovator's product were yellow since it was the color of piroxicam. Over the time of studying, there were no changes in color as inspected visually. These might be due to (1) they were stable, (2) the color changes was so slightly that it could not be seen visually since the original color of the formulas was yellow.

4.2 Chemical Stability.

The rate constants obtained from the concentration-time profiles (zero-order kinetics), and the log (concentration) - time profiles (first-order kinetics) of piroxicam injections at 65 c ,55 c, and 45 c were presented in Table 19 and 20. The data in both Tables indicated that the rate constants showed a positive correlation with the increasing temperture. It might be due to the higher temperature activated more decomposition of piroxicam in solution than the lower temperature since the number of collision increases as the temperature increases (Connors, Amidon and Kennon,1986).

The rate constants at 32 c (calculated by Arrhenius method) were presented in Table 21. It was seen that formulation No.1 and No. 5 had the highest rate constant. Because both formulas did not contain any added additive resulting in degradation processes proceeded faster than others. However, of all the formulas with added

Table 19 : The rate constants (k) of piroxicam injection formulation no. 1,2,3,4, at 65° C' 55° C, 45° C

Formula No	temp (°C)	Zero-order (k_0) (mg ml^{-1} day^{-1})[a]			First-order (k_1) (day^{-1})[b]		
		run 1	run 2	run 3	run 1	run 2	run 3
1	65°	0.03595	0.04224	0.03903	0.00197	0.00229	0.00208
	55°	0.02325	0.02368	0.02511	0.00124	0.00123	0.00132
	45°	0.02164	0.01903	0.02175	0.00116	0.00100	0.00114
2	65°	0.05172	0.05357	0.05807	0.00298	0.00309	0.00336
	55°	0.03475	0.03612	0.03343	0.00197	0.00202	0.00184
	45°	0.00944	0.01079	0.01175	0.00051	0.00058	0.00063
3	65°	0.05141	0.04761	0.05657	0.00288	0.00270	0.00316
	55°	0.03375	0.03055	0.03360	0.00184	0.00168	0.00180
	45°	0.01267	0.01120	0.01121	0.00067	0.00060	0.00058
4	65°	0.04248	0.04435	0.04462	0.00239	0.00249	0.00251
	55°	0.01582	0.01572	0.01424	0.00084	0.00083	0.00075
	45°	0.00752	0.00804	0.00800	0.00039	0.00042	0.00042

[a] k_0 was obtained from the slope of concentration vs time curve

[b] k_1 was obtained from the slope of log (concentration) vs time curve

Table 20 : The rate constant (k) of formulated piroxicam injection formulation No. 5, 6, 7, 8 and innovator's product at 65°C, 55°C, and 45°C

Formula No	temp (°C)	Zero-order (k_0) (mg ml^{-1} day^{-1})[a]			First-order (k_1) (day^{-1})[b]		
		run 1	run 2	run 3	run 1	run 2	run 3
5	65°	0.03901	0.04127	0.04453	0.00219	0.00235	0.00250
	55°	0.03284	0.02883	0.02958	0.00178	0.00158	0.00159
	45°	0.02143	0.01961	0.02545	0.00117	0.00107	0.00137
6	65°	0.04701	0.04552	0.04786	0.00268	0.00253	0.00264
	55°	0.03485	0.03390	0.03426	0.00193	0.00185	0.00183
	45°	0.01015	0.01206	0.01113	0.00054	0.00064	0.00057
7	65°	0.05167	0.05044	0.05610	0.00290	0.00278	0.00306
	55°	0.03400	0.03724	0.03798	0.00185	0.00200	0.00203
	45°	0.01067	0.01194	0.01298	0.00056	0.00062	0.00067
8	65°	0.04662	0.04655	0.04429	0.00259	0.00256	0.00249
	55°	0.02293	0.02299	0.01970	0.00122	0.00120	0.00106
	45°	0.00809	0.01096	0.00901	0.00042	0.00056	0.00048
Innovator's product	65°	0.04218	0.04523	0.04568	0.00228	0.00242	0.00245
	55°	0.01529	0.01484	0.01616	0.00078	0.00075	0.00082
	45°	0.00804	0.00785	0.00784	0.00040	0.00039	0.00039

[a] k_0 was obtained from the slope of concentration vs time curve

[b] k_1 was obtained from the slope of log (concentration) vs time curve

Table 21 : The rate constants of piroxicam injection at room temperature (32°C) calculated by Arrhenius method.

Formula No.	Zero-order (k_0) (mg ml^{-1} day^{-1})[a]			First-order (k_1) (day^{-1})[b]		
	run1	run2	run3	run1	run2	run3
1	0.01416	0.01009	0.01359	0.00074	0.00051	0.00070
2	0.00316	0.00382	0.00398	0.00016	0.00020	0.00020
3	0.00499	0.00427	0.00379	0.00025	0.00022	0.00019
4	0.00206	0.00220	0.00211	0.00010	0.00011	0.00010
5	0.01443	0.01148	0.01630	0.00077	0.00060	0.00085
6	0.00385	0.00515	0.00436	0.00020	0.00027	0.00021
7	0.00373	0.00477	0.00488	0.00019	0.00024	0.00025
8	0.00239	0.00386	0.00283	0.00012	0.00019	0.00014
innovator's product	0.00228	0.00205	0.00208	0.00011	0.00010	0.00010

substances, formula No. 4 with sodium sulfite in combination of benzyl alcohol had the lowest rate constant.Having these two substances might exert the stability of piroxicam injection.

The correlation coefficients between the concentration-time and the log (concentration)-time of all formulas were shown in Table 22 and 23. These values were all significant at 95 % level of probability. After simulating the data for zero and first order kinetics, both cases showed the values of the correlation coefficient closed to -1 for each formula. Therefore,it could not be concluded that the kinetic reactions of piroxicam in all formulas were either first or zero order kinetics.

The variables of Arrhenius equation of all piroxicam formulations were shown in Table 24 . The activation energy of most formulas, except those of formula No. 1 and No. 5 , were in normal range which was about 12 to 24 K cal/ mol (Connors, Amidon and Kennon , 1986). In this study , formulation No.4 had the highest activation energy (Table 24) and the lowest rate constant (Table 19 and 20). Thus, formulation No.4 seemed to be the most stable formula.

The average shelf-lives at room temperature (32°C) calculated from the predicted rate constants were presented in Table 25.Results indicated that the shelf-life of these formulas were varied upon the presence of additives in the formulas (formulas No 1 and 5 had no additives,

Table 22 : The correlation coefficient (r) obtained from the concentration vs time profiles (zero-order reaction) and log (concentration) vs time profiles(first-order reaction) of formulation No.1,2,3 and 4.

Formula No	temp (°C)	r of zero-order			r from first order		
		run1	run2	run3	run1	run2	run3
1	65°	-0.9522	-0.9769	-0.9954	-0.9527	-0.9808	-0.9966
	55°	-0.9907	-0.9936	-0.8989	-0.9905	-0.9948	-0.9042
	45°	-0.9164	-0.9306	-0.9921	-0.9159	-0.9358	-0.9934
2	65°	-0.9847	-0.9977	-0.9872	-0.9842	-0.9978	-0.9884
	55°	-0.9903	-0.9812	-0.9992	-0.9878	-0.9799	-0.9994
	45°	-0.9254	-0.9328	-0.9109	-0.9262	-0.9333	-0.9115
3	65°	-0.9487	-0.9201	-0.9603	-0.9438	-0.9134	-0.9528
	55°	-0.9406	-0.9849	-0.9734	-0.9422	-0.9861	-0.9754
	45°	-0.9436	-0.9861	-0.9860	-0.9461	-0.9872	-0.9871
4	65°	-0.9491	-0.9506	-0.9644	-0.9446	-0.9459	-0.9599
	55°	-0.9612	-0.9703	-0.9753	-0.9626	-0.9712	-0.9758
	45°	-0.9906	-0.9896	-0.9741	-0.9909	-0.9897	-0.9744

Table 23 : The correlation coefficient obtained from the concentration vs time profiles (zero-order reaction) and log (concentration) vs time profiles (first-order reaction) of formulation No.5-8 and innovator's product.

Formula No	temp (°C)	r of zero-order			r from first order		
		run1	run2	run3	run1	run2	run3
5	65°	-0.8878	-0.9622	-0.8794	-0.8971	-0.9685	-0.8919
	55°	-0.9775	-0.9975	-0.9487	-0.9791	-0.9981	-0.9533
	45°	-0.9187	-0.8208	-0.9223	-0.9232	-0.8233	-0.9248
6	65°	-0.9744	-0.9798	-0.9608	-0.9699	-0.9782	-0.9664
	55°	-0.9867	-0.9800	-0.9928	-0.9874	-0.9785	-0.9916
	45°	-0.9840	-0.9826	-0.9782	-0.9849	-0.9825	-0.9794
7	65°	-0.9933	-0.9872	-0.9895	-0.9904	-0.9864	-0.9867
	55°	-0.9963	-0.9877	-0.9730	-0.9965	-0.9877	-0.9717
	45°	-0.9877	-0.9871	-0.9587	-0.9878	-0.9858	-0.9598
8	65°	-0.9877	-0.9847	-0.9910	-0.9872	-0.9841	-0.9880
	55°	-0.9847	-0.9526	-0.9994	-0.9861	-0.9537	-0.9996
	45°	-0.9518	-0.9758	-0.9808	-0.9531	-0.9763	-0.9814
Innovator's product	65°	-0.9890	-0.9412	-0.9751	-0.9899	-0.9390	-0.9761
	55°	-0.9863	-0.9781	-0.9558	-0.9853	-0.9785	-0.9581
	45°	-0.9963	-0.9291	-0.9826	-0.9961	-0.9293	-0.9829

Table 24 : The variables of Arrhenius equation of piroxicam formulation.

Formulation No.	Zero-Order		First-Order	
	log A	Ea (k Cal / mol)	log A	Ea (k Cal / mol)
1	2.89	6.69 ± 1.60	1.78	6.93 ± 1.66
2	10.12	17.53 ± 0.64	9.37	18.29 ± 0.59
3	9.08	15.97 ± 1.22	8.27	16.65 ± 1.34
4	10.45	18.32 ± 0.14	9.71	19.11 ± 0.20
5	2.99	6.77 ± 1.05	2.00	7.17 ± 1.08
6	8.73	15.47 ± 1.12	7.93	16.15 ± 1.26
7	9.14	15.90 ± 0.75	8.31	16.89 ± 1.08
8	9.70	17.07 ± 1.65	8.89	17.76 ± 1.62
Innovator's product	10.49	18.37 ± 0.61	9.75	19.19 ± 0.56

Table 25 : Shelf-life at room temperature (32°C) calculated from the predicted rate constants

pH	Formula No.	Shelf-Life (Zero Order) Predicted (Day)	Shelf-Life (First Order) Predicted (Day)
7.5	1	160.00 ± 32.51	164.67 ± 34.30
	2	522.67 ± 60.45	565.67 ± 64.74
	3	449.00 ± 65.55	486.33 ± 77.26
	4	906.00 ± 28.51	1010.33 ± 39.02
7.0	5	139.67 ± 23.03	144.67 ± 25.38
	6	439.67 ± 61.10	476.33 ± 72.82
	7	445.33 ± 62.94	472.00 ± 69.86
	8	667.00 ±151.70	727.33 ± 162.51
pH 8	Innovator's product	948.45 ± 60.62	1054.12 ± 55.59

formulas No. 2 and 7 were added with sodium sulfite 0.15% w/v as an antioxidant, formulas 3 and 6 were added with 2% benzyl alcohol as an antimicrobial, and formulas No.4 and 8 were added with both additives). Hence, in order to test for the effects of the additives on the shelf-life of all formulated piroxicam injections , a One-Way Analysis of Variance was performed (Milton and Arnold, 1990). Results were shown in Tables 26-29 .

It was clearly seen that there were statistically significant differences ($p < 0.05$) in shelf-lives among the formulas in each pH group. Then Least Significant Difference (LSD) was used to determine whether which mean of shelf-life was different from that of control (comparing between added-additive formula with no additive formula) (Milton & Arnold, 1990). From the test (Table 30) using $LSD_{(0.05)}$, it implied that the formulas with added substances had longer shelf-life than that without such materials. This could be concluded that use of sodium sulfite or benzyl alcohol alone or use of both in combination could stabilize at pH 7 and pH 7.5 piroxicam injections.

The best formula of pH 7 and pH 7.5 were selected on the basis of their shelf-lives. The representation from pH 7 was formula 8 with shelf-life of 667 days (zero-order) and 727 days (first -order) meanwhile that from pH 7.5 was formula 4 with shelf-life of 906 days (zero-order) and 1010 days (first-order). Both formulas

Table 26 : The statistical analysis of shelf-life from various formulations of pH 7.5 by using one-way ANOVA.

Formulation No.	Shelf-life from zero order (day) 1	2	3	4
	136	592	385	934
	197	495	446	877
	147	481	516	907
Total	480	1568	1347	2718
Mean	160	522.67	449	906

ANOVA Table				
Source	df[a]	SS[b]	MS[c]	V.R.[d]
Among groups	3	849,588.25	283,196.08	115.34
Within groups	8	19,642.67	2,455.33	
Total	11			

$F_{0.05(3,8)} = 4.07$

[a] = degree of freedom

[b] = sum square

[c] = mean square = SS/df

[d] = variance ratio

Table 27 : The statistical analysis of shelf-life from various formulations of pH7 by using one-way ANOVA

Formulation No.	Shelf-life from zero order (day) 5	6	7	8
	134	493	518	814
	165	373	410	511
	120	453	408	676
Total	419	1319	1336	2001
Mean	139.67	439.67	445.33	667

ANOVA Table				
Source	df[a]	SS[b]	MS[c]	V.R.[d]
Among groups	3	421,770.917	140,590.31	18.00
Within groups	8	62,476	7,809.50	
Total	11			

$F_{0.05\ (3,8)} = 4.07$

[a] = degree of freedom [b] = sum square

[c] = mean square = SS/df [d] = variance ratio

Table 28 : The statistical analysis of shelf-life from various formulations of pH 7.5 by using one-way ANOVA

Formulation No.	Shelf-life from first order (day)			
	1	2	3	4
	141	640	413	1050
	204	535	479	972
	149	522	567	1009
Total	494	1697	1459	3031
Mean	164.67	565.67	486.33	1010.33

ANOVA Table				
Source	df[a]	SS[b]	MS[c]	V.R.[d]
Among groups	3	1,093,515.58	364,505.19	113.43
Within groups	8	25,708.67	3,213.58	
Total	11			

$F_{0.05(3,8)} = 4.07$

[a] = degree of freedom [b] = sum square

[c] = mean square = SS/df [c] = variance ratio

Table 29 The statistical analysis of shelf-life from various formulations of pH7 by using one-way ANOVA

Formulation No.	Shelf-life from first order (day)			
	5	6	7	8
	137	538	552	889
	173	396	441	564
	124	495	423	729
Total	434	1429	1416	2182
Mean	144.67	476.33	472	727.33

	ANOVA Table			
Source	df[a]	SS[b]	MS[c]	V.R.[d]
Among groups	3	513,648.92	171,216.33	18.39
Within groups	8	74,472.00	9,309.00	
Total	11			

$F_{0.05\ (3,8)} = 4.07$

[a] = degree of freedom [b] = sum square

[c] = mean square = SS/df [d] = variance ratio

Table 30: The Least Significant Difference (LSD) value of shelf-lives of the formulated piroxicam injections.

Zero-order shelf-life		First-order shelf-life	
Δ-value (days)	LSD(0.05)	Δ-value (days)	LSD(0.05)
362.67(X_2-X_1)	93.30	401.11(X_2-X_1)	106.74
289.00(X_3-X_1)	93.30	321.66(X_3-X_1)	106.74
746.00(X_4-X_1)	93.30	845.66(X_4-X_1)	106.74
300.00(X_6-X_5)	166.39	331.66(X_6-X_5)	181.66
305.66(X_7-X_5)	166.39	327.33(X_7-X_5)	181.66
527.33(X_8-X_5)	166.39	582.66(X_8-X_5)	181.66

$t_{0.05,\ df\ 8}$ = 2.306

Δ-value = different between two means.

X_n = mean of shelf-life of formulation No.n.

LSD = $t_{0.05,8}$ $\underline{2MSE}$ (n = 3)

(formulas 4 and 8) contained sodium sulfite and benzyl alcohol. A t-test was used to test for the difference of the shelf-life of these two formulas (Table 31). Results in Table 31 showed that no statistical difference ($p > 0.05$) between the zero- ordered shelf-lives of formula 4 and formula 8. On the other hand the first-ordered shelf-lives of these two formulas did show significant difference ($p<0.05$). Thus, between formulas 4 and 8, formula 4 with pH 7.5 appeared to be the better formula since it had longer shelf-life. In another word, it could be concluded that between formulas 4 and 8, the formula with higher pH (formula 4) was more stable, and was the best formula of all.

To compare the formulated injection with that of the innovator's product, formula 4 which was the best of all the formulated products was chosen as a representative. Again at-test was used in the same way as before (Table 31). It was seen in Table 31 that the shelf-lives of both formulations were not statistical different from each other. This might be due to the two formulas contained almost the same added substances.

Even the shelf-life of formula 4 was not significantly different from that of the innovator's product,it was also needed some improvement. First, the amount of nicotinamide should be reduced for prevention of some side effects that might be associated. Second, the solubility of the formula should be increased since there was some crystals occured during storage in the

Table 31: The t-statistical value in comparison of shelf-lives.

Formulas to compare	t-statistical values	
	Zero-order	First-order
No.4 (pH 7.5) and No.8 (pH 7.0)	2.68 (NS)	2.93 (S)
No.4 (pH 7.5) and Innovator's product	1.09 (NS)	1.20 (NS)

$t_{0.05,4}$ = 2.776

NS = non significant ($p>0.05$)

S = significant ($p<0.05$)

refrigerator. The solubility of this formula could be increased by increasing the pH of the formula to about 8 to be similar to that of the innovator's product. Using a high quality of the piroxicam raw material might improve drug solubility as well. If the solubility increased, the amount of nicotinamide could be reduced too. Third, in this study sodium sulfite and benzyl alcohol were added to preserve potency of the formula. The effects of these excipients, at normally used concentrations, were studied by comparing the formulas with and without the excipients. The optimal concentrations of the excipients used in the formulas had not been studied yet.

Therefore, if this piroxicam injection formula needed to be prepared in the manufacturing drug industry, it should be improved following all the above discussion. Finally, it is hoped that this study will serve as a guide line for a good formulation of piroxicam injection.