CHAPTER IV

RESULTS AND DISCUSSION

A. Formulation of ketoprofen in liquid vehicle

Hard gelatin capsule is suitable for dispensing liquid such as oil, paraffins, silicones or thickening agents. These activities concentrated on the formulation of the capsule contents into paste having a consistency which sufficient to withstand leakage from the unseal capsule. The disadvantage of pastes is the viscous which affect to weight uniformity and bioavailability problems. Thus, in this study the formulation of the content for capsule filling was conformed in solution by using cosolvents which was alternative way to minimize these problems.

Selection of liquid vehicle: PEGs are widely used in a variety of pharmaceutical formulations including parenteral, topical, opthalmic, oral and rectal preparations. All grades of PEG are soluble in water. Liquid PEG may cause hardening of capsule shell by preferential absorption of moisture from gelatin in the shell. They are very hygroscopic although hygroscopicity decreases with increasing molecular weight. However, solid grades, e.g. PEG 4000 and those with higher molegular weight are not hygroscopic (Kibbe, 2000). Additionally, PEG with higher molegular weight is also having high melting point, which affect to prepare the formulation. In this study, PEG 1500 (melting point 44 - 48^{o}C) was used as a high component of cosolvent because of its suitable melting point for preparing the formulation. PG has also been widely used as a solvent in parenteral and nonparenteral pharmaceutical formulations. It is used to dissolve water insoluble drugs, such as corticosteroids.

Surfactant is a common substance that is used to enhance drug dissolution from solid, liquid and semi-solid dosage form including suppositories. Surfactants are classified into various groups based on their structures. In this experiment, some of them were selected to incorporate as liquid base. Group of polyoxyethylene sorbitan fatty acid

ester (Tween) is the most widely used surfactant in liquid preparations as well as in suppository formulations in order to enhance drug release from suppository dosage form (Babar et al, 1999). DMI is a solvent with low hemolytic potential (Reed and Yalkowsky, 1985) and is used as cosolvent for several nonpolar drugs.

From preliminary study, for solubilization of ketoprofen 100 mg by using the combination of two cosolvents PEG 1500 and PG, Tween 60® or Tween 80® as cosolvent, ketoprofen was precipitated in all proportions of 150 mg and 200 mg of cosolvent. But 100 mg of ketoprofen was solubilized when using PEG 1500 in combination with DMI in proportion of 1:9 and 9:1 for 150 mg and 200 mg of cosolvent , respectively.

In this study, the combination of three cosolvents (PEG 1500, PG and A*) and physical appearance of liquid formulas of ketoprofen are shown in Tables 3 to 5. It was found that the precipitation of ketoprofen was seen in every proportions of 100 mg cosolvent (Table 2). The solubility of the drug was increased by using 150 and 200 mg of cosolvent (Tables 3 - 4). Various proportions of solvent system with Tween 60® or Tween 80® were not able to dissolve the drug when temperature was changed. However, the precipitation was decreased by using DMI in solvent system. Because DMI could improve drug solubility with decreasing dielectric constant of solvent system (Zia et al, 1991).

Nine formulations were selected for further *in vitro* studies according to the criteria specified earlier. Formulations 1 to 3 were those with Tween 60®, formulations 4 to 6 were those with Tween 80® and the formulations 7 to 9 were those with DMI.

All liquid formulas were prepared on the weight by weight (W/W) basis, by varying the ratio of cosolvent. The amount of ingredients used in each formula is present in Table 5. The liquid of each formula equivalent to 300 mg was transferred into each capsule body No.O and its individual cap was then secured.

Table 2. Physical appearance of liquid formula of 100 mg. ketoprofen in 100 mg. of cosolvent

The amount of cosolvent (mg) (PEG1500 : PG : A*)	A * = Tween 60®				A * = Tween 80®				A * = DMI			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
80:10:10	-	-	-	-	-	-	-	-	-	-	-	-
70:10:20	-	-	-	-	-	-	-	-	-	-	-	-
70:20:10	-	-	-	-	-	-	-	-	-	-	-	-
60:10:30	-	-	-	-	-	-	-	-	-	-	-	-
60:20:20	-	-	-	-	-	-	-	-	-	-	-	-
60:30:10	-	-	-	-	-	-	-	-	-	-	-	-
50:10:40	-	-	-	-	-	-	-	-	-	-	-	-
50:20:30	-	-	-	-	-	-	-	-	-	-	-	-
50:30:20	-	-	-	-	-	-	-	-	-	-	-	-
50:40:10	-	-	-	-	-	-	-	-	-	-	-	-
40:10:50	-	-	-	-	-	-	-	-	-	-	-	-
40:20:40	-	-	-	-	-	-	-	-	-	-	-	-
40:30:30	-	-	-	-	-	-	-	-	-	-	-	-
40:40:20	-	-	-	-	-	-	-	-	-	-	-	-
40:50:10	-	-	-	-	-	-	-	-	-	-	-	-
30:10:60	-	-	-	-	-	-	-	-	-	-	-	-
30:20:50	-	-	-	-	-	-	-	-	-	-	-	-
30:30:40	-	-	-	-	-	-	-	-	-	-	-	-
30:40:30	-	-	-	-	-	-	-	-	-	-	-	-
30:50:20	-	-	-	-	-	-	-	-	-	-	-	-
30:60:10	-	-	-	-	-	-	-	-	-	-	-	-
20:10:70	-	-	-	-	-	-	-	-	-	-	-	-
20:60:60	-	-	-	-	-	-	-	-	-	-	-	-
20:30:50	-	-	-	-	-	-	-	-	-	-	-	-
20:40:40	-	-	-	-	-	-	-	-	-	-	-	-
20:50:30	-	-	-	-	-	-	-	-	-	-	-	-
20:60:20	-	-	-	-	-	-	-	-	-	-	-	-
20:70:10	-	-	-	-	-	-	-	-	-	-	-	-
10:10:80	-	-	-	-	-	-	-	-	-	-	-	-
10:20:70	-	-	-	-	-	-	-	-	-	-	-	-
10:30:60	-	-	-	-	-	-	-	-	-	-	-	-
10:40:50	-	-	-	-	-	-	-	-	-	-	-	-
10:50:40	-	-	-	-	-	-	-	-	-	-	-	-
10:60:30	-	-	-	-	-	-	-	-	-	-	-	-
10:70:20	-	-	-	-	-	-	-	-	-	-	-	-
10:80:10	-	-	-	-	-	-	-	-	-	-	-	-

Table 3. Physical appearance of liquid formula of 100 mg. Ketoprofen in 150 mg. of cosolvent

The amount of cosolvent (mg) (PEG1500 : PG : A*)	A * = Tween 60®				A * = Tween 80®				A * = DMI			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
120:15:15	+	+	-	-	+	+	-	-	+	-	-	-
105:15:30	+	+	-	-	+	+	-	-	+	+	+	+
105:30:15	+	+	-	-	+	-	-	-	+	-	-	-
90:45:15	+	-	-	-	+	-	-	-	+	+	+	+
90:30:30	+	-	-	-	+	-	-	-	+	-	-	-
90:45:15	+	+	-	-	+	+	-	-	+	-	-	-
75:15:60	+	-	-	-	+	-	-	-	+	+	+	+
75:30:45	+	+	-	-	+	+	-	-	+	+	+	+
75:45:30	+	+	-	-	+	+	-	-	+	-	-	-
75:60:15	+	+	-	-	+	+	-	-	+	-	-	-
60:15:75	+	-	-	-	+	+	-	-	+	+	+	+
60:30:60	+	-	-	-	+	+	+	+	+	+	+	+
60:45:45	+	+	-	-	+	-	-	-	+	+	+	+
60:60:30	+	-	-	-	+	+	-	-	+	+	-	-
60:75:15	+	+	-	-	+	+	-	-	+	+	-	-
45:15:90	+	-	-	-	+	+	-	-	+	+	+	+
45:30:75	+	-	-	-	+	-	-	-	+	+	+	+
45:45:60	+	+	+	-	+	+	-	-	+	+	+	+
45:60:45	+	+	-	-	+	+	-	-	+	+	+	+
45:75:30	+	-	-	-	+	+	-	-	+	+	-	-
45:90:15	+	+	-	-	+	-	-	-	+	+	-	-
30:15:105	+	+	-	-	+	-	-	-	+	+	+	+
30:30:90	+	+	-	-	+	-	-	-	+	+	+	+
30:45:75	+	+	-	-	+	-	-	-	+	+	+	+
30:30:60	+	-	-	-	+	+	-	-	+	+	+	+
30:75:45	+	-	-	-	+	+	-	-	+	+	+	+
30:90:30	+	-	-	-	+	+	-	-	+	+	-	-
30:105:15	+	-	-	-	+	+	-	-	+	+	-	-
15:15:120	-	-	-	-	+	+	-	-	+	+	+	+
15:30:105	+	+	-	-	+	-	-	-	+	+	+	+
15:45:90	+	+	-	-	+	+	-	-	+	+	+	+
15:60:75	+	-	-	-	+	+	-	-	+	+	+	+
15:75:60	+	-	-	-	+	+	-	-	+	+	+	+
15:90:45	+	-	-	-	+	+	-	-	+	+	-	-
15:105:30	+	+	-	-	+	+	-	-	+	+	-	-
15:120:15	+	-	-	-	+	+	-	-	+	+	-	-

Table 4. Physical appearance of liquid formula of 100 mg. Ketoprofen in 200 mg. of cosolvent

The amount of cosolvent (mg) (PEG1500 : PG : A*)	A * = Tween 60®				A * = Tween 80®				A * = DMI			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
160:20:20	+	+	+	+	+	+	+	+	+	+	+	+
140:20:40	+	+	+	+	+	+	+	+	+	+	+	+
140:40:20	+	+	+	+	+	+	+	+	+	-	-	-
120:20:60	+	+	+	+	+	+	+	+	+	+	+	+
120:40:40	+	+	+	+	+	+	+	+	+	+	+	+
120:60:20	+	+	+	+	+	+	+	+	+	+	+	+
100:20:80	+	+	+	+	+	+	+	+	+	+	+	+
100:40:60	+	+	+	+	+	+	+	+	+	+	+	+
100:60:40	+	-	-	-	+	+	+	+	+	+	+	+
100:80:20	+	-	-	-	+	+	+	+	+	+	+	+
80:20:100	+	+	+	+	+	+	+	+	+	+	+	+
80:40:80	+	+	+	+	+	+	+	+	+	+	+	+
80:60:60	+	-	-	-	+	+	+	+	+	+	+	+
80:80:40	+	+	+	+	+	+	+	+	+	+	+	+
80:100:20	+	-	-	-	+	+	-	-	+	+	+	+
60:20:120	+	+	+	+	+	+	+	+	+	+	+	+
60:40:100	+	-	-	-	+	+	+	+	+	+	+	+
60:60:80	+	+	+	+	+	+	+	+	+	+	+	+
60:80:60	+	-	-	-	+	+	+	+	+	+	+	+
60:100:40	+	-	-	-	+	+	+	+	+	+	+	+
60:120:20	+	+	+	+	+	+	-	-	+	+	+	+
40:20:140	+	+	+	+	+	+	+	+	+	+	+	+
40:40:120	+	+	+	+	+	+	+	+	+	+	+	+
40:60:100	+	+	+	+	+	+	+	+	+	+	+	+
40:80:80	+	+	+	+	+	-	-	-	+	+	+	+
40:100:60	+	-	-	-	+	+	+	+	+	+	+	+
40:120:40	+	+	+	+	+	+	-	-	+	+	+	+
40:140:20	+	-	-	-	+	+	-	-	+	+	+	+
20:20:160	+	+	+	+	+	-	-	-	+	+	+	+
20:40:140	+	+	+	+	+	-	-	-	+	+	+	+
20:60:120	+	+	+	+	+	-	-	-	+	+	+	+
20:80:100	+	+	+	+	+	+	-	-	+	+	+	+
20:100:80	+	-	-	-	+	+	-	-	+	+	+	+
20:120:60	+	+	+	+	+	+	+	-	+	+	+	+
20:140:40	+	-	-	-	+	-	-	-	+	-	-	-
20:160:20	+	-	-	-	+	-	-	-	+	-	-	-

I = after mixing liquid by vortexing for 5 minutes

II = after storage at room temperature for 24 hours

III = after storage in refrigerator for 24 hours

IV = after storage at room temperature for 24 hours

\+ = clear solution

\- = precipitate

Table 5. The formulation of ketoprofen liquid-filled hard gelatin capsule

Formula	Ketoprofen (mg)	% w/w of cosolvent (200 mg)				
		PEG 1500	PG	Tween 60®	Tween 80®	DMI
1	100	80	10	10	-	-
2	100	70	10	20	-	-
3	100	60	10	30	-	-
4	100	80	10	-	10	-
5	100	70	10	-	20	-
6	100	60	10	-	30	-
7	100	80	10	-	-	10
8	100	70	10	-	-	20
9	100	60	10	-	-	30

B. *In Vitro* studies

1. Release characteristics of ketoprofen from hard gelatin capsule

Effect of Tween 60®, Tween 80® and DMI on release profiles: Tween 60®, Tween 80® and DMI provided the same release pattern of the drug. The release profiles were shown in Figures 6 to 8. Drug released was increased when the concentration of Tween 60®, Tween 80® and DMI were increased. Within 30 minutes percent release of ketoprofen with 10%, 20% and 30% Tween 60® were 43.44%, 56.45% and 57.82%

whereas those with the same concentration of Tween 80® were 53.82%, 57.20% and 59.48%, respectively (Tables 6 - 7). The percent ketoprofen released was increased with increasing concentration of surfactant. This effect arise from surfactant could improve the drug release by increasing wettability and reducing interfacial tension of the system following increase of surfactant concentration. These results were consistent to the previous report (Dredan et al., 1985). The percent release of ketoprofen at 30 minutes from all formulations with DMI were found to be less than 50% as displayed in Table 8. The effect of dimethyl isosorbide on release profile was to increase drug release with undergoes complexation with water through hydrogen bonding interaction. The solvent complex exhibited a major effect on the solubility of drug (Zia et al., 1991).

The release characteristics of ketoprofen from hard gelatin capsules in the present study appeared to be the same with respect to the pattern of dissolution profile from ketoprofen tablet in the previous study. Percent release of ketoprofen from tablet was approximately 67% at 30 minutes under the same conditions (Maffione et al., 1993).

2. Selection of the best formulation

According to the criteria specified earlier the formula with 10% of Tween 80® and that with 10 % of DMI should be selected for further study but those with 20% of Tween 80® and 20% of DMI were easier to prepare than the formulae mentioned above. Thus, the formula with 20% of Tween 80® and another was that with 20 % of DMI were selected for further *in vivo* study.

Both formulations were freshly prepared again and then these capsules were sealed and coated, respectively. To seal the hard gelatin capsule after filling with liquid was to eliminate the risk of leakage during storage at elevated temperatures. The coated capsule using HPMC showed good gliding excellent slip when contacted to water. These coated hard gelatin capsules were suitable for rectal application.

Table 6. Percent released of ketoprofen (Mean ± S.D.) from three formulations of ketoprofen rectal capsule with Tween 60®

Time (min)	% Released (Mean ± S.D.)		
	10% Tween 60®	20% Tween 60®	30% Tween 60®
5	7.725 ± 2.109	14.824 ± 4.042	14.809 ± 3.818
10	16.680 ± 2.199	26.324 ± 5.526	26.200 ± 5.725
15	24.630 ± 3.332	37.779 ± 5.861	36.795 ± 5.302
20	33.538 ± 3.001	44.258 ± 5.137	45.236 ± 4.681
30	43.438 ± 4.768	56.447 ± 4.684	57.819 ± 4.777
45	55.848 ± 5.697	70.033 ± 3.923	71.906 ± 4.904
60	68.410 ± 6.008	79.858 ± 3.261	80.805 ± 4.124
80	79.839 ± 5.350	89.092 ± 2.581	90.137 ± 3.010
100	89.039 ± 4.044	93.520 ± 1.516	94.268 ± 2.130
120	93.906 ± 2.876	94.866 ± 0.772	96.639 ± 1.482

N = 12

Table 7. Percent released of ketoprofen (Mean ± S.D.) from three formulations of ketoprofen rectal capsule with Tween 80®

Time (min)	% Released (Mean ± S.D.)		
	10% Tween 80®	20% Tween 80®	30% Tween 80®
5	12.426 ± 1.994	15.787 ± 5.588	17.058 ± 8.874
10	23.816 ± 3.287	27.796 ± 6.149	29.309 ± 6.550
15	33.517 ± 4.679	38.571 ± 6.318	39.000 ± 6.000
20	40.821 ± 4.327	46.009 ± 5.939	46.435 ± 5.681
30	53.822 ± 3.032	57.199 ± 6.044	59.480 ± 5.464
45	66.646 ± 3.239	70.531 ± 5.902	74.400 ± 5.121
60	77.289 ± 2.801	81.159 ± 5.144	84.609 ± 4.601
80	86.675 ± 2.021	90.409 ± 3.890	92.968 ± 3.761
100	93.182 ± 1.399	95.589 ± 3.250	96.502 ± 2.664
120	95.694 ± 1.112	98.396 ± 2.244	97.667 ± 1.851

N = 12

Table 8. Percent released of ketoprofen (Mean ± S.D.) from three formulations of ketoprofen rectal capsule with DMI

Time (min)	% Released (Mean ± S.D.)		
	10% DMI	20% DMI	30% DMI
5	4.145 ± 0.812	5.336 ± 1.345	5.940 ± 1.414
10	10.797 ± 1.121	13.548 ± 2.541	14.910 ± 3.742
15	17.114 ± 1.921	21.040 ± 3.457	22.685 ± 5.848
20	23.152 ± 2.633	27.457 ± 4.174	29.068 ± 6.817
30	33.352 ± 3.474	37.163 ± 5.431	40.610 ± 8.514
45	47.315 ± 4.573	51.089 ± 7.139	53.395 ±10.611
60	59.384 ± 5.852	62.540 ± 9.259	66.797 ± 12.568
80	74.905 ± 7.300	75.209 ± 10.092	77.498 ± 10.214
100	84.080 ± 7.413	82.314 ± 8.672	84.168 ± 7.982
120	89.655 ± 6.242	86.996 ± 7.174	89.315 ± 5.844

N = 12

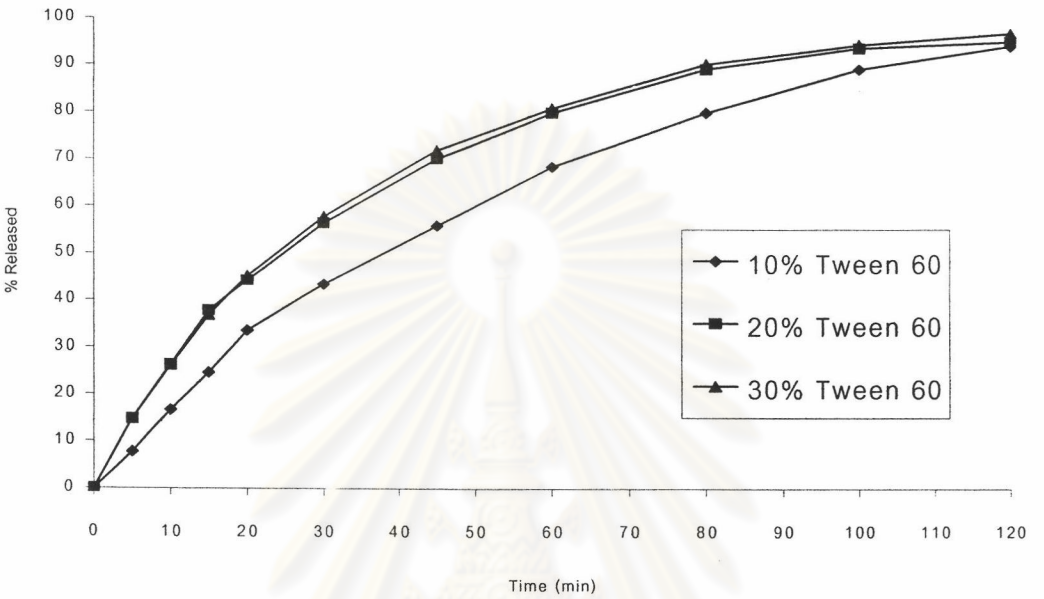


Figure 6. Dissolution profile of ketoprofen from three formulations of ketoprofen rectal capsule with Tween 60®

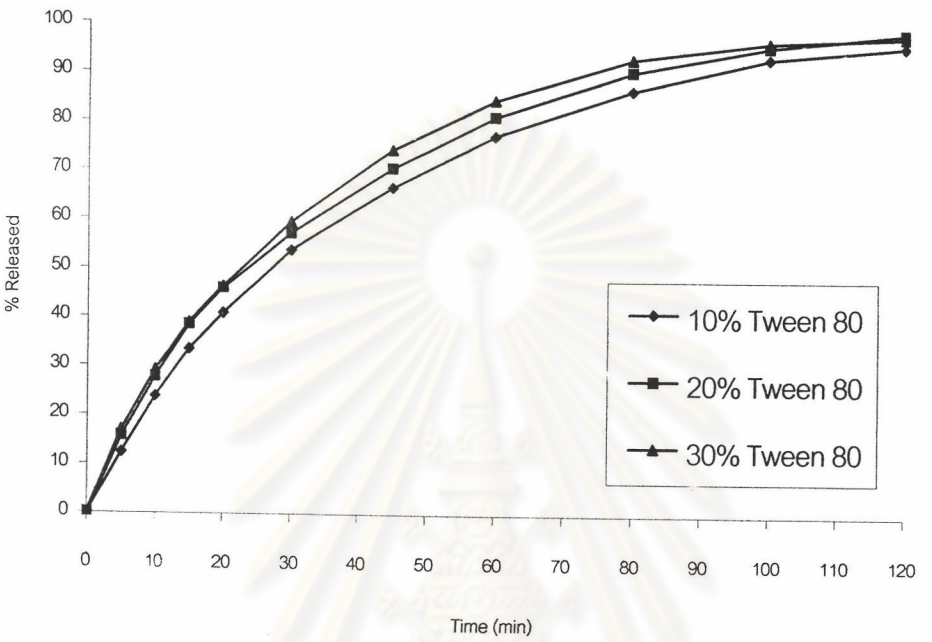


Figure 7. Dissolution profile of ketoprofen from three formulations of ketoprofen rectal capsule with Tween 80®

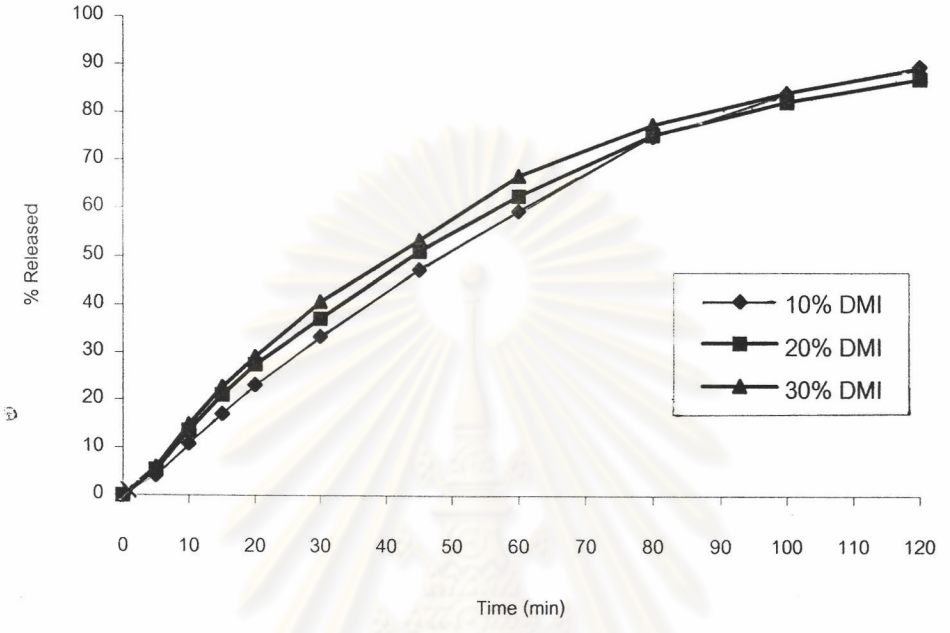


Figure 8. Dissolution profile of ketoprofen from three formulations of ketoprofen rectal capsule with DMI

However, liquid-filled hard gelatin capsule containing high concentration of surfactant might cause removal of water from the protein structure of capsule with resulting in loss of mechanical strength (Cole et al., 1992). DMI was miscible in all proportion with water thus DMI might cause to affect the integrity of the gelatin shell. Hence, the amount of surfactant and DMI employed to improve release of drug in each preparation should be used at lowest concentration.

C. Evaluation of physical and chemical properties of coated ketoprofen rectal capsule

1.Dissolution profile of coated capsule

Comparative release characteristics of ketoprofen between uncoated and coated capsule were shown in Figures 9 to 10. The release profile of all coated capsules exhibited a characteristic lag time. This was seen by drug release began after 15 minutes (Tables 9-10). The capsules coated with HPMC (Methocel® E5), when contacted with dissolution medium, the polymeric coating layer swelled to form a viscous barrier and spent some times before allowing drug release. However, dissolution profile of coated capsule after lag time was similar to that of uncoated capsules. These results agree with previous report (Maffione et al., 1993). It was reported that the release profile of coated ketoprofen tablets showed a characteristic lag time, the lag time increased as a function of amount of HPMC in coating layer. Additionally, the high viscosity of HPMC retard drug release with slightly changing in the pattern of dissolution profile.

2. Disintegration time

The results of disintegration of uncoated and coated rectal capsule from 20% Tween 80® formulation and 20% DMI formulation were shown in Tables 11 to 12, respectively. It was found that the disintegration time of hard gelatin capsule uncoated and coated with HPMC were between 3 – 5 minutes. The gelatin shell ruptures allowing release of the contents within 30 minutes. The comparative disintegration of uncoated and coated rectal capsule from both preparations is slightly different.

3. Content uniformity

The uniformity of content of ketoprofen in each coated rectal capsule preparation stated as percent labeled amount (% L.A.) was shown in Table13. They were 101.95 ± 1.38 percent for 20% Tween 80® formulation and 101.72 ± 1.14 percent for 20% DMI formulation. Each of them met the BP 1993 specification within the range of 92.5 - 107.5% of the labeled claim. The BP specifies the content uniformity standard for suppositories, each of 10 dosage unit as determined from the content uniformity method lies within the range of 85 – 115% of the label claim.

D. Stability of coated ketoprofen rectal capsule

1. Physical appearance

The coated capsules were stored at 40°C with 75% RH for three months. All coated capsules were still in good physical appearance. Cracking, wrinkling or tearing of capsule shell was not visually observed but color of coated film and liquid system became darken (Figure11). The color of liquid system from 20% Tween 80® formula was darken than that with 20% DMI. This could be due to Tween 80® was sensitive to oxidation. Additionally, PEG 1500 exposure to heat might induce oxidation (Kibbe, 2000). There was no visually observable physical interaction between liquid content and the gelatin capsules.

2. Content of ketoprofen (% L.A.)

The method of analysis was validated by determining the accuracy, the within run and between run precision. Results are shown in Appendix C. The accuracy in term of percent recovery for all concentrations was between 97.59 – 99.28%. The within run and between run precision expressed as percent coefficient of variations were 0.66 – 0.85% and 0.87 - 1.63%, respectively. The calibration curve of PAR of ketoprofen to

Table 9. Percent released of ketoprofen (Mean ± S.D.) from 20% Tween 80® formulation

Time (min)	% Released (Mean ± S.D.)	
	Uncoated capsule	Coated capsule
5	3.311 ± 3.054	*
10	13.698 ± 10.225	*
15	30.843 ± 5.022	7.203 ± 9.960
20	41.241 ± 5.083	16.144 ± 14.365
30	53.676 ± 4.661	38.495 ± 13.561
45	70.319 ± 3.659	59.215 ± 11.637
60	81.040 ± 2.297	75.537 ± 9.034
80	89.662 ± 2.387	85.997 ± 6.968
100	95.066 ± 1.350	93.488 ± 1.722
120	97.041 ± 0.797	96.420 ± 0.854

N = 12

* = could not measure

Table 10. Percent released of ketoprofen (Mean ± S.D.) from 20% DMI formulation

Time (min)	% Released (Mean ± S.D.)	
	Uncoated capsule	Coated capsule
5	0.598 ± 0.570	*
10	3.407 ± 1.876	*
15	8.677 ± 3.169	2.644 ± 1.467
20	16.166 ± 4.660	5.739 ± 3.498
30	29.816 ± 6.118	19.619 ± 4.774
45	46.158 ± 8.056	36.490 ± 6.945
60	61.635 ± 12.921	49.397 ± 9.193
80	74.561 ± 11.558	63.093 ± 10.667
100	84.023 ± 9.448	72.768 ± 11.446
120	89.813 ± 7.807	79.939 ± 11.139

N = 12

* = could not measure

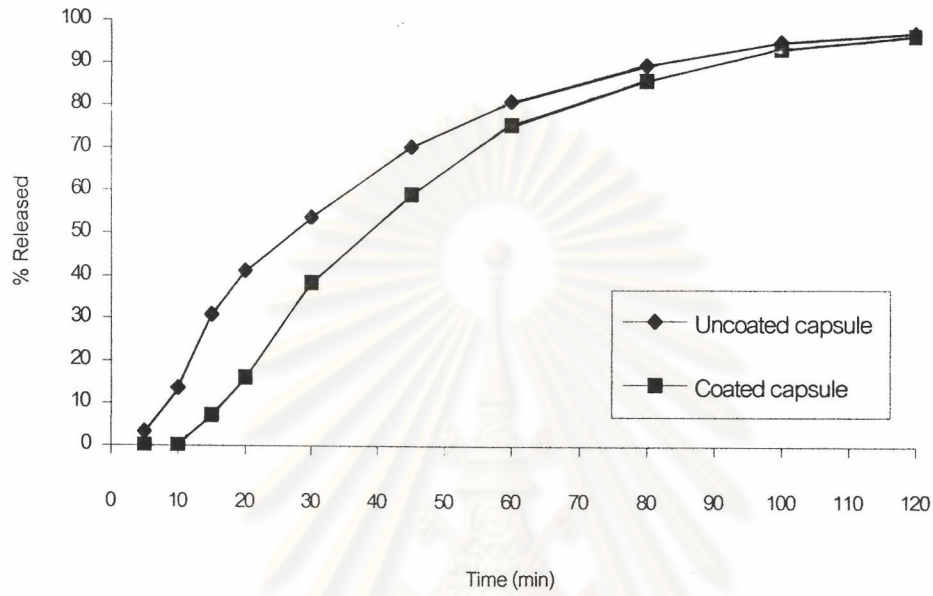


Figure 9. Dissolution profile of ketoprofen from uncoated and coated capsules with 20% Tween 80® formulation

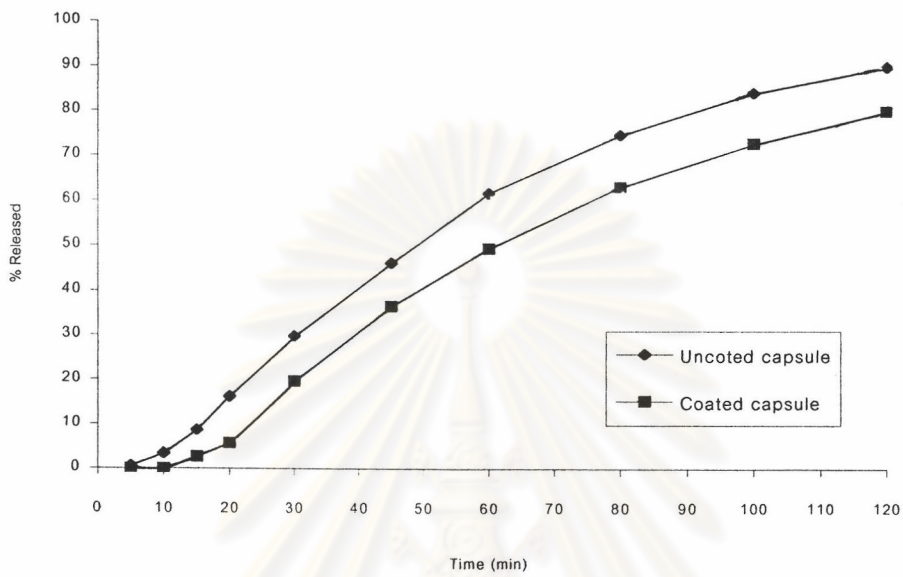


Figure 10. Dissolution profile of ketoprofen from uncoated and coated capsules with 20% DMI formulation

Table 11. Disintegration time of uncoated and coated rectal capsule in water at 37 ± 2°C from 20% Tween 80® formulation

Sample no.	Disintegration time (min)	
	Uncoated capsule	Coated capsule
1	3.73	3.95
2	3.08	4.37
3	3.72	4.87
4	2.88	5.08
5	3.37	4.20
6	4.00	4.16
Mean	3.40	4.43
S.D.	0.43	0.44

Table 12. Disintegration time of uncoated and coated rectal capsule in water at 37 ± 2°C from 20% DMI formulation

Sample no.	Disintegration time (min)	
	Uncoated capsule	Coated capsule
1	2.92	3.75
2	3.33	3.97
3	2.97	3.37
4	3.42	4.25
5	2.25	3.60
6	3.67	4.00
Mean	3.09	3.82
S.D.	0.50	0.31

Table 13. Uniformity of content of ketoprofen (% L.A.) from 20% Tween 80® formulation and 20% DMI formulation

Rectal capsule no.	% L.A. of ketoprofen	
	20% Tween 80®	20% DMI
1	103.60	102.39
2	100.26	102.69
3	103.60	100.26
4	100.87	101.32
5	100.56	102.84
6	100.87	99.95
7	103.45	102.69
8	102.08	102.84
9	101.02	101.63
10	103.15	100.56
Mean	101.95	101.72
S.D.	1.38	1.14

diclofenac sodium versus ketoprofen concentrations was linear covering all concentrations determined with the coefficient of determination of 1.0.

The result of assay of ketoprofen content in coated capsules prior to and after storage in the condition specified above showed that percent drug content remained in the range of 92.5 – 107.5%, which still met the BP 1993 specifications. The average contents from time zero up to three months were shown in Tables 14 - 15.

The content of ketoprofen was changed. This could be due to high temperature and moisture in storage condition (75% RH). Additionally, the carbonyl and ester groups in ketoprofen structure were sensitive to hydrolysis. All of these were waiting to be proven in the future.

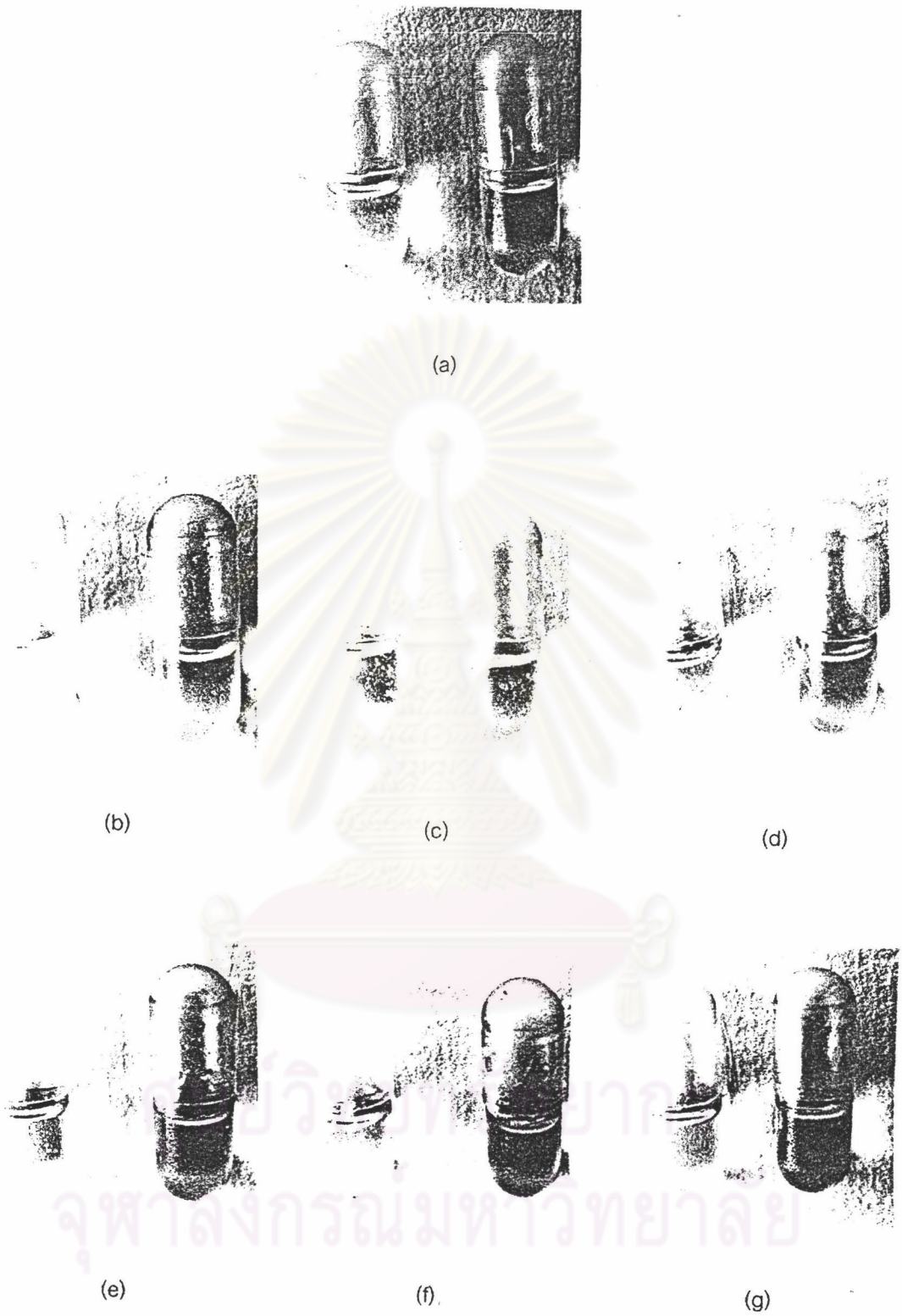


Figure 11. Physical appearance of coated ketoprofen rectal capsule at 40°C with 75% RH; (left) 20% DMI formulation, (right) 20% Tween 80® formulation, (a) 0 month, (b) 0.5 month, (c) 1 month, (d) 1.5 month, (e) 2 month, (f) 2.5 month, (g) 3 month

Table 14. Content of ketoprofen (% L.A.) from 20% Tween 80® formulation at 40°C with 75% RH, for three months

Capsule No.	Time (month)						
	0	0.5	1	1.5	2	2.5	3
1	99.554	100.638	97.753	95.375	95.161	93.556	92.379
2	99.782	98.733	98.154	95.056	94.279	94.208	93.359
3	100.811	99.948	98.316	95.215	95.220	93.630	92.546
4	98.335	98.755	97.294	95.336	94.225	93.010	91.956
5	100.612	97.820	98.853	96.248	95.649	93.063	93.035
6	100.15	97.585	97.879	95.792	96.562	93.121	91.896
Mean	99.784	98.913	98.041	95.503	95.183	93.431	92.529
S.D.	0.892	1.189	0.532	0.439	0.878	0.462	0.583

Table 15. Content of ketoprofen (% L.A.) from 20% DMI formulation at 40°C with 75% RH, for three months

Capsule No.	Time (month)						
	0	0.5	1	1.5	2	2.5	3
1	100.437	100.408	97.295	96.595	96.011	95.479	93.487
2	100.699	99.361	98.059	95.959	96.058	95.271	92.197
3	100.320	100.114	97.304	96.277	96.180	95.787	93.802
4	101.101	100.421	98.970	97.009	96.796	93.711	91.652
5	100.457	99.652	99.290	98.048	96.125	93.564	93.724
6	101.612	99.860	98.251	97.528	95.883	94.965	93.577
Mean	100.771	99.969	98.195	96.903	96.009	94.796	93.073
S.D.	0.497	0.424	0.828	0.786	0.146	0.938	0.913

E. *In Vivo* studies

One hundred milligram of ketoprofen in coated rectal capsules with 20% Tween 80® and with 20% DMI were prepared to be used for *in vivo* studies. They were tested and found to conform for uniformity of content of BP 1993.

1. Analysis of ketoprofen concentrations in rabbit plasma

Figure 12 showed chromatograms of blank rabbit plasma, rabbit plasma spiked with ketoprofen and internal standard, and plasma sample taken at 1 hours post dose from a rabbit following administration of 100 mg ketoprofen in coated rectal capsule. Ketoprofen and diclofenac sodium were well separated from endogenous substance peak with the retention times of about 6.31 and 9.58 min, respectively.

The method of analysis was validated by determining the accuracy, the within run and between run precision. Results were accessible in Appendix D. Accuracy in term of percent recovery for all concentrations was between 102.50 – 108.94%. Within run and between run precision expressed as percent coefficient of variations were 2.01 - 4.73% and 2.59 - 3.76%, respectively. Calibration curve of PAR of ketoprofen to diclofenac sodium versus plasma ketoprofen concentrations was linear cover all concentration tested with the coefficient of determination of 0.9999.

2. Plasma ketoprofen concentrations

Plasma ketoprofen concentrations at any sampling time interval up to 12 hours from 12 rabbits after rectal administration of two formulations of ketoprofen in coated rectal capsule and an Oruvail® were presented in Tables 16 to 18. Comparisons of the plasma ketoprofen concentration-time profiles of each rabbit were illustrated in Figures 13 to 24 and all profiles were summarized for 12 rabbits graphically in Figure 25. As seen, from individual plot, some profiles exhibited irregular post absorption phase and more than one peak were demonstrated. This was probably predominant due to

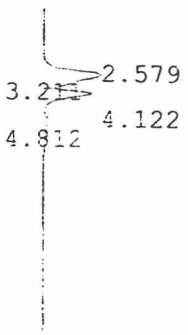
subject variations since it was very difficult to administer the formulation into individual rectally. However, this was not observed from average profiles because all data were taken into accounted resulted in reducing of variability.

3. Pharmacokinetics analysis

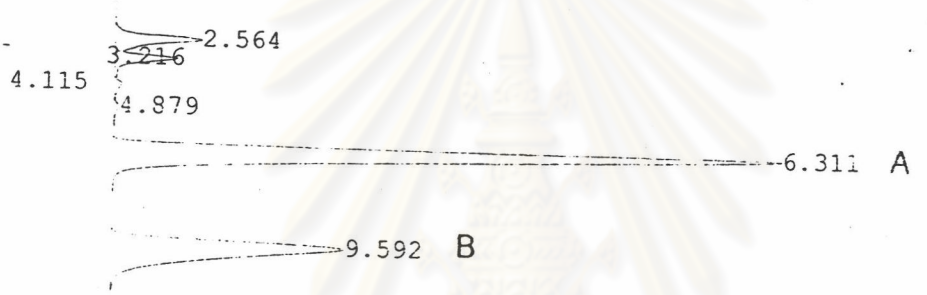
Generally, pharmacokinetic study is the most recommended method for measuring product quality bioavailability and establish bioequivalence. This may be viewed as a bioassay that assesses release of the drug substance from the formulation into the systemic circulation. In pharmacokinetic study, physiological variables are assumed to have less interoccasion variability compared to the variability arising from formulation performance. Thus, differences between two products due to formulation factors can be determined.

3.1 Comparison of pharmacokinetic parameters

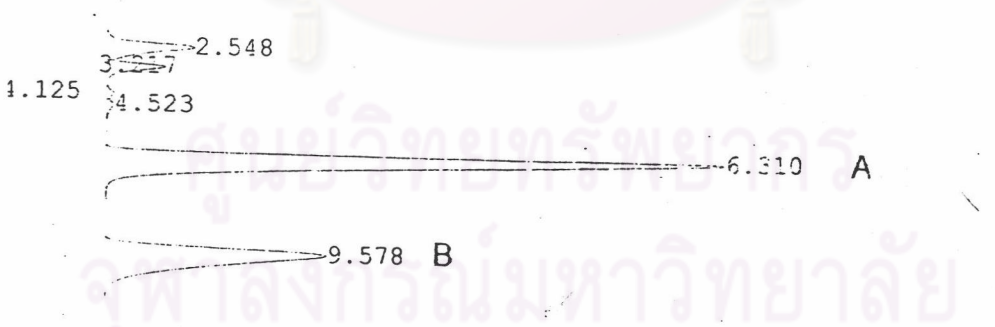
All relevant pharmacokinetic parameters were obtained and only the corresponding parameters of the two formulations of 100 mg ketoprofen coated rectal capsule were compared. They were summarized as follows:



Blank rabbit plasma.



Spike rabbit plasma (ketoprofen conc. 240 μg/mL).



Rabbit plasma taken at 1 hour post dose (ketoprofen observed conc. 149.45 μg/mL).

Figure 12. High performance liquid chromatographic peaks of ketoprofen (A) and diclofenac sodium (B)

Table 16. Plasma ketoprofen concentration (μg/mL) of twelve rabbits after administration of 100 mg ketoprofen in coated rectal capsule with 20% Tween 80® formulation

Rabbit	Time (hr)										
No.	0.5	1	1.5	2	2.5	3	4	6	8	10	12
1	142.966	236.123	181.761	150.616	131.473	128.983	86.911	34.002	31.768	17.611	10.014
2	166.063	114.776	86.646	78.518	64.359	55.925	51.406	28.442	17.773	9.257	2.680
3	246.541	172.903	191.416	164.425	108.952	113.374	80.337	54.036	33.158	24.785	19.612
4	288.993	229.515	182.104	160.085	136.536	115.837	80.632	61.028	51.292	30.246	26.146
5	312.352	221.862	144.604	123.002	130.406	101.006	80.017	52.836	37.700	29.779	19.503
6	191.966	218.767	155.098	116.602	103.891	99.638	83.820	32.794	31.851	17.823	10.081
7	107.510	174.633	203.094	181.118	170.222	156.185	116.712	77.829	40.353	16.703	9.883
8	229.586	200.971	201.631	145.421	156.687	126.213	109.514	54.102	30.867	20.349	11.266
9	247.637	215.550	172.928	138.401	132.343	104.804	84.545	53.351	36.085	24.454	19.941
10	119.932	233.435	179.331	160.477	93.089	107.279	74.552	51.609	30.531	14.234	8.860
11	171.366	235.907	211.619	169.888	116.939	120.028	66.620	57.998	31.370	12.980	5.578
12	101.597	244.240	173.455	153.900	140.400	119.705	77.045	46.230	37.504	26.514	13.531
Mean	193.876	208.224	173.640	145.204	123.775	112.415	82.675	50.355	34.188	20.395	13.091
S.D.	70.930	37.468	33.487	28.014	28.588	23.497	17.193	13.633	7.814	6.764	6.850

Table 17. Plasma ketoprofen concentration (μg/mL) of twelve rabbits after administration of 100 mg ketoprofen in coated rectal capsule with 20% DMI formulation

Rabbit	Time (hr)										
No.	0.5	1	1.5	2	2.5	3	4	6	8	10	12
1	139.864	132.792	108.414	90.191	88.547	98.193	56.772	48.806	19.063	15.005	20.053
2	98.520	173.430	137.541	100.345	85.413	86.744	65.014	30.287	16.740	8.952	4.328
3	135.863	79.330	61.657	40.850	39.028	34.532	17.694	17.530	12.030	7.619	4.272
4	215.472	256.093	220.960	191.754	132.038	187.167	124.938	76.322	65.282	54.554	40.192
5	207.536	237.603	153.964	97.641	116.191	130.293	81.472	39.654	27.921	13.702	9.843
6	153.657	180.144	146.537	108.381	100.006	59.340	78.717	38.568	28.257	13.950	9.958
7	169.112	286.025	246.602	108.773	140.172	143.787	78.552	58.930	21.313	12.970	7.951
8	225.091	227.073	191.033	155.159	140.062	141.100	118.674	58.081	39.981	34.413	25.918
9	133.734	72.129	74.301	48.497	47.278	39.414	25.009	13.072	7.202	5.560	3.265
10	166.987	212.183	165.669	170.744	136.644	137.446	98.704	65.673	40.211	15.407	8.667
11	145.271	180.811	118.706	110.029	91.408	97.169	75.540	35.702	17.927	8.300	3.457
12	164.678	170.028	91.784	72.139	64.548	50.553	39.508	27.516	18.679	10.386	5.053
Mean	162.982	178.720	143.097	113.875	98.445	100.478	71.716	42.512	26.217	16.735	11.913
S.D.	37.338	69.380	56.841	50.344	35.666	48.396	33.544	19.462	15.874	14.009	11.304

Table 18. Plasma ketoprofen concentration (μg/mL) of twelve rabbits after intramuscular administration of 50 mg ketoprofen (Oruvail®)

Rabbit	Time (hr)										
No.	0.5	1	1.5	2	2.5	3	4	6	8	10	12
1	53.273	50.154	46.019	41.374	40.228	37.102	35.795	28.017	21.085	14.958	11.782
2	70.684	79.737	72.150	63.427	62.052	47.020	39.332	26.171	9.455	4.157	2.938
3	56.561	50.526	44.754	38.464	38.395	34.950	34.972	27.542	21.277	14.243	8.871
4	64.604	67.894	64.340	59.504	41.119	37.219	26.703	11.101	4.017	2.952	2.370
5	79.688	84.754	60.666	58.848	54.336	40.706	33.671	21.591	11.976	10.917	7.244
6	68.938	65.964	68.648	62.833	58.756	49.982	44.010	31.200	19.907	17.799	11.273
7	79.172	74.725	64.628	57.021	50.690	40.150	28.030	11.826	4.220	2.971	2.384
8	52.412	66.733	58.644	49.994	44.984	47.114	35.727	28.953	19.383	18.546	12.782
9	49.083	70.019	62.510	44.635	38.672	42.339	27.466	19.258	13.177	7.506	5.260
10	84.534	90.640	87.872	80.778	63.698	47.629	53.454	21.740	13.507	7.735	5.391
11	45.142	56.147	48.702	46.984	39.195	33.138	24.945	7.338	2.941	2.835	2.595
12	71.724	87.938	70.818	51.346	50.424	31.446	24.779	5.489	3.366	2.044	2.405
Mean	64.651	70.436	62.479	54.601	48.546	40.733	34.074	20.019	12.026	8.889	6.275
S.D.	13.159	13.740	12.236	11.690	9.453	6.173	8.596	8.984	7.240	6.946	5.401

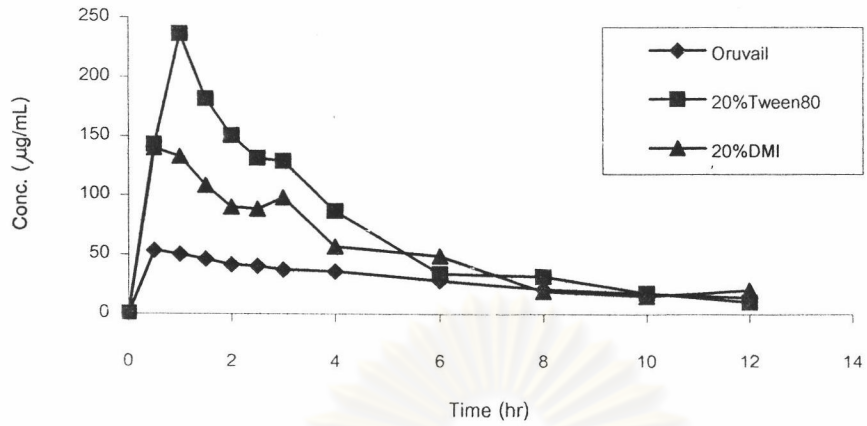


Figure 13. Plasma ketoprofen concentration-time curve of rabbit No.1 after rectal administration of two formulations of 100 mg ketoprofen (20%Tween80® formulation and 20%DMI formulation) and intramuscular administration of 50 mg Oruvail®

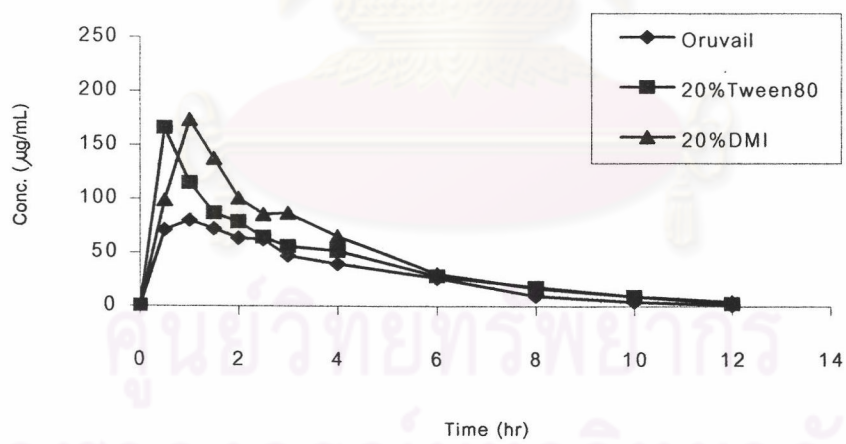


Figure 14. Plasma ketoprofen concentration-time curve of rabbit No.2 after rectal administration of two formulations of 100 mg ketoprofen (20%Tween80® formulation and 20%DMI formulation) and intramuscular administration of 50 mg Oruvail®

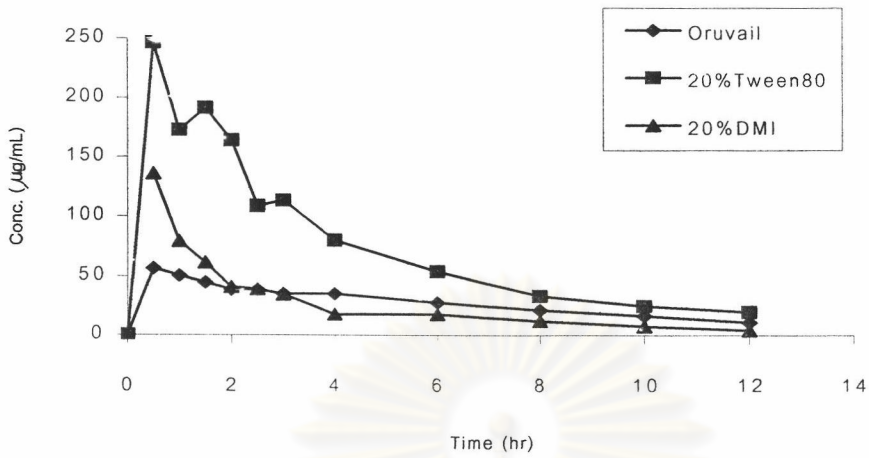


Figure 15. Plasma ketoprofen concentration-time curve of rabbit No.3 after rectal administration of two formulations of 100 mg ketoprofen (20%Tween80® formulation and 20%DMI formulation) and intramuscular administration of 50 mg Oruvail®

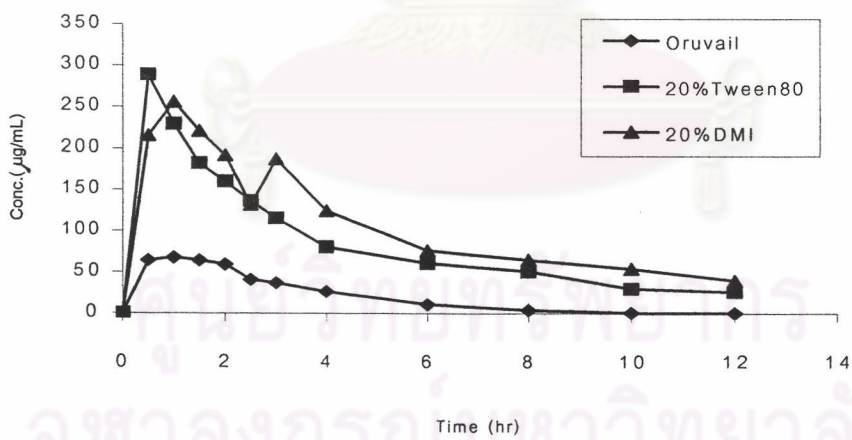


Figure 16. Plasma ketoprofen concentration-time curve of rabbit No.4 after rectal administration of two formulations of 100 mg ketoprofen (20%Tween80® formulation and 20%DMI formulation) and intramuscular administration of 50 mg Oruvail®

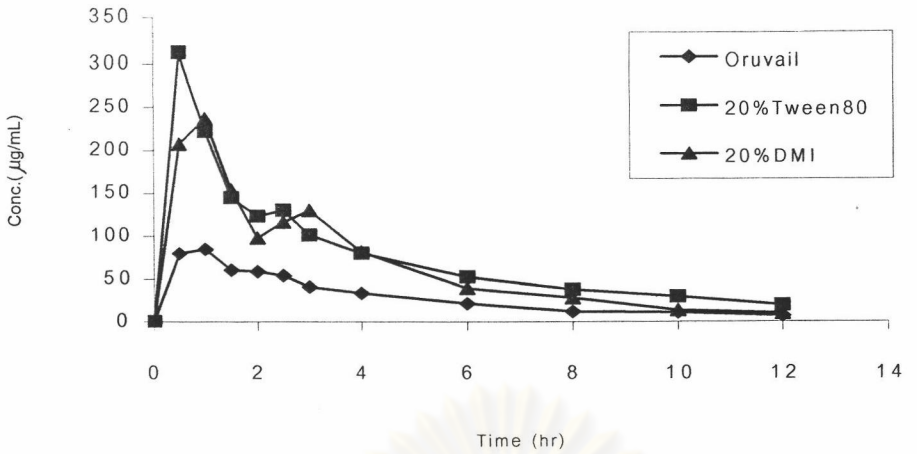


Figure 17. Plasma ketoprofen concentration-time curve of rabbit No.5 after rectal administration of two formulations of 100 mg ketoprofen (20%Tween80® formulation and 20%DMI formulation) and intramuscular administration of 50 mg Oruvail®

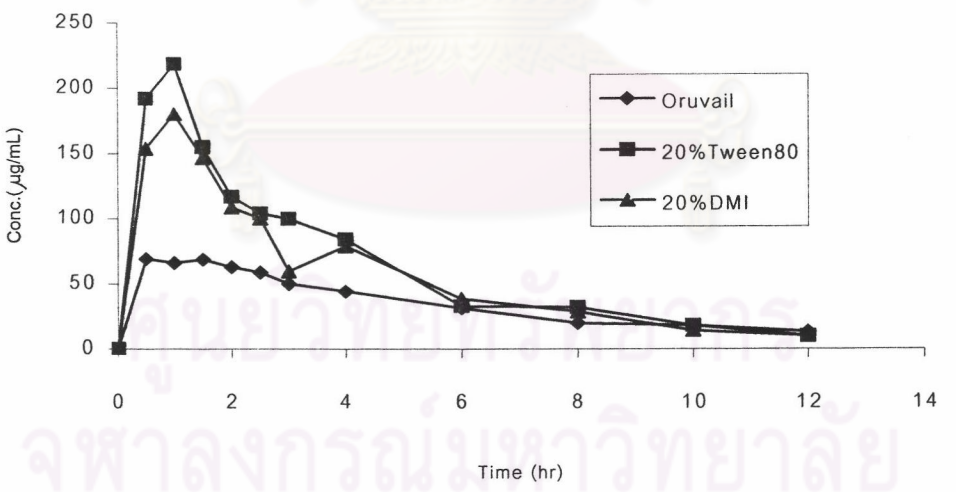


Figure 18. Plasma ketoprofen concentration-time curve of rabbit No.6 after rectal administration of two formulations of 100 mg ketoprofen (20%Tween80® formulation and 20%DMI formulation) and intramuscular administration of 50 mg Oruvail®

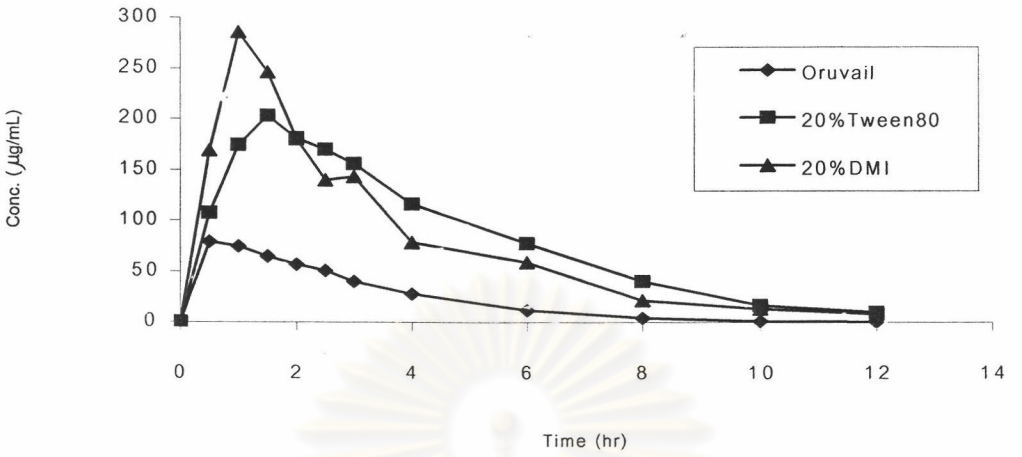


Figure 19. Plasma ketoprofen concentration-time curve of rabbit No.7 after rectal administration of two formulations of 100 mg ketoprofen (20%Tween80® formulation and 20%DMI formulation) and intramuscular administration of 50 mg Oruvail®

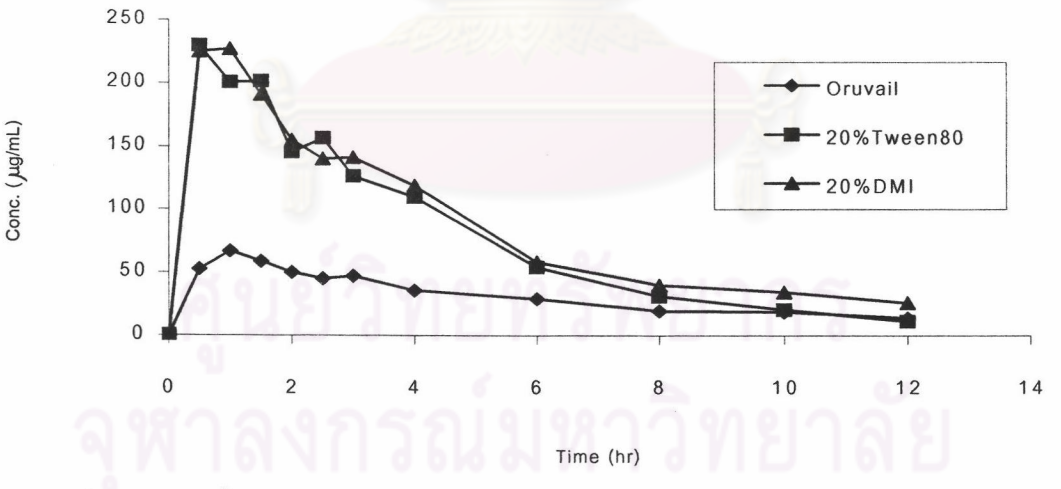


Figure 20. Plasma ketoprofen concentration-time curve of rabbit No.8 after rectal administration of two formulations of 100 mg ketoprofen (20%Tween80® formulation and 20%DMI formulation) and intramuscular administration of 50 mg Oruvail®

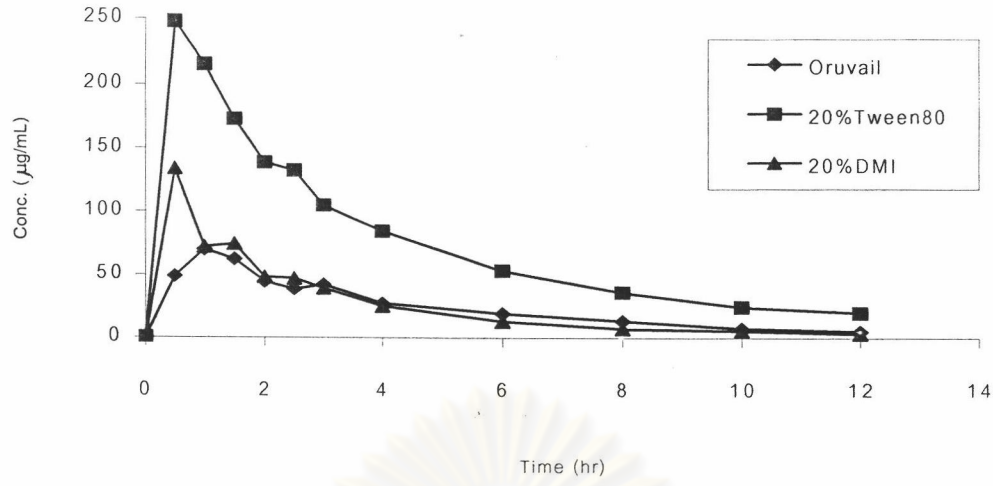


Figure 21. Plasma ketoprofen concentration-time curve of rabbit No.9 after rectal administration of two formulations of 100 mg ketoprofen (20%Tween80® formulation and 20%DMI formulation) and intramuscular administration of 50 mg Oruvail®

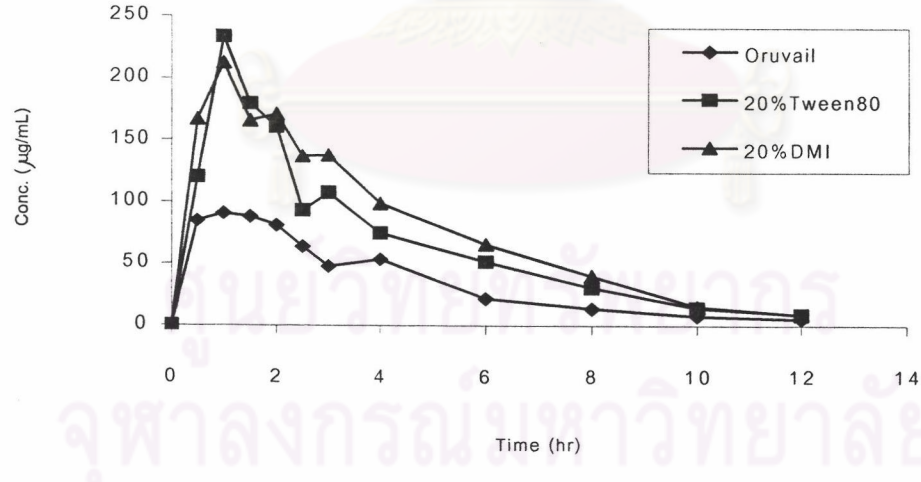


Figure 22. Plasma ketoprofen concentration-time curve of rabbit No.10 after rectal administration of two formulations of 100 mg ketoprofen (20%Tween80® formulation and 20%DMI formulation) and intramuscular administration of 50 mg Oruvail®

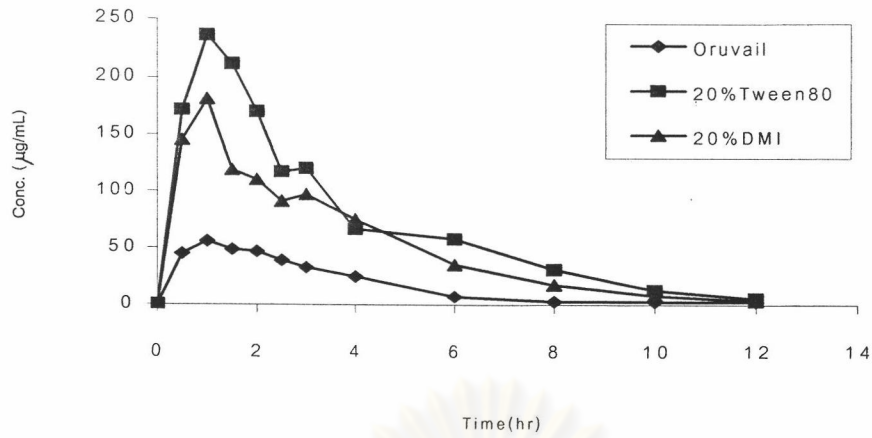


Figure 23. Plasma ketoprofen concentration-time curve of rabbit No.11 after rectal administration of two formulations of 100 mg ketoprofen (20%Tween80® formulation and 20%DMI formulation) and intramuscular administration of 50 mg Oruvail®

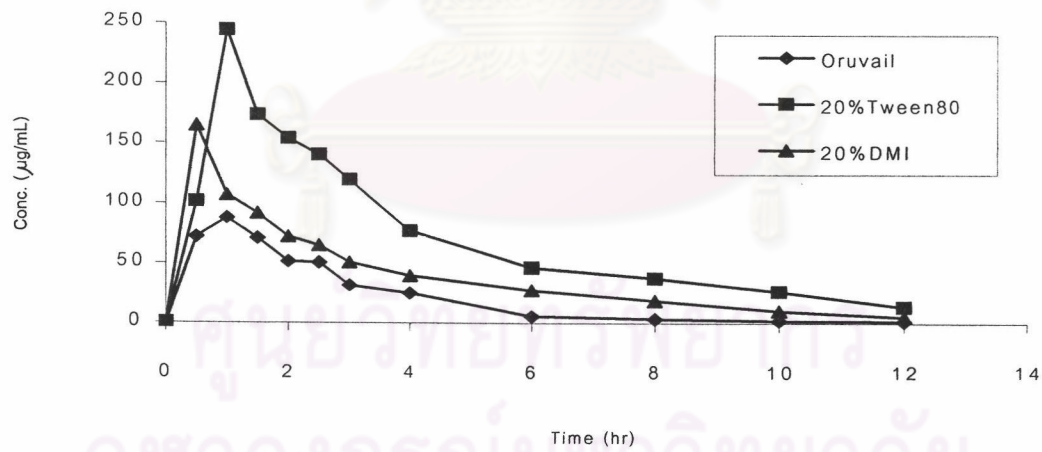


Figure 24. Plasma ketoprofen concentration-time curve of rabbit No.12 after rectal administration of two formulations of 100 mg ketoprofen (20%Tween80® formulation and 20%DMI formulation) and intramuscular administration of 50 mg Oruvail®

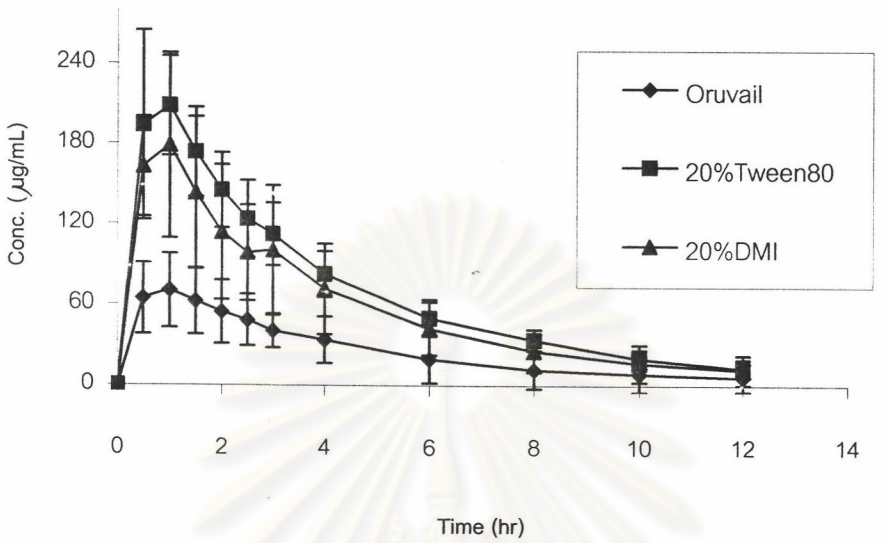


Figure 25. Comparison of Plasma ketoprofen concentration-time curve (mean ± S.D.) of twelve rabbits after rectal administration of two formulations of 100 mg ketoprofen (20% Tween80® formulation and 20% DMI formulation) and intramuscular administration of 50 mg Oruvail®

Peak plasma ketoprofen concentration (C_{max})

The C_{max} (Mean $\pm$ S.D.) of ketoprofen obtained from the two formulations of coated rectal capsules with 20% Tween 80®, 20% DMI and Oruvail® were 238.562 $\pm$ 37.163, 193.958 $\pm$ 49.764 and 71.817 $\pm$ 12.697μg/mL (Table 19), respectively. As observed, this parameter was more variable than others which was anticipated although crossover study was employed. The C_{max} value from the formula with 20% Tween 80® appeared to be higher than that with 20% DMI. This was expected since Tween 80® could improve drug absorption by reducing interfacial tension of the system (Ken et al., 1993). Result in Tables 20 showed that there were statistically significant difference ($p < 0.05$) between the C_{max} values of the two test formulations for formulation effect. This indicated intra-subject variability. This difference could be due to small number of subjects participating in the experiment.

Time to peak plasma ketoprofen concentration (t_{max})

The onset of drug was indicated by this parameter. Both test formulations as well as Oruvail® were rapidly absorbed as seen by the peak plasma concentration was reached to a maximum value within a short period of time apparently from 0.5 – 1 hr, as shown in Table 21 indicating the drug from all formulations were regularly and steadily absorbed. The t_{max} (Mean $\pm$ S.D.) of ketoprofen from coated rectal capsules with 20% Tween 80®, 20% DMI and Oruvail® were 0.79 $\pm$ 0.33, 0.83 $\pm$ 0.25 and 0.83 $\pm$ 0.25 hour, respectively. These values were approximately the same as those reported previously (Barba et al., 1999; Nilufer and Ermis, 1996). There were no statistically significant differences ($p>0.05$) between t_{max} values of the two test formulations (Table 22). This referred that the time required to attain the peak plasma concentration for all formulations were markedly the same.

Table 19. Peak plasma concentrations (C_{max}) of ketoprofen of twelve rabbits after rectal administration of two formulations of 100 mg ketoprofen and intramuscular administration of 50 mg Oruvail®

Rabbit No.	C_{max} (μg/mL)		
	20 % Tween 80®*	20 % DMI*	Oruvail®**
1	236.123	139.864	53.273
2	166.063	173.430	79.737
3	246.541	135.863	56.561
4	288.993	256.093	67.894
5	312.352	237.603	84.754
6	218.767	180.144	68.938
7	203.094	286.025	79.172
8	229.586	227.073	66.733
9	247.637	133.734	70.019
10	233.435	212.183	90.640
11	235.907	180.811	56.147
12	244.240	164.678	87.938
Mean	238.562	193.958	71.817
S.D.	37.163	49.764	12.697

* 100 mg

** 50 mg

Table 20. Analysis of variance for C_{max} after rectal administration of two formulations of 100 mg ketoprofen (α=0.05)

Source of variation	d.f.	SS	MS	F ratio	F table	Sig.level
Total	23	54370.28	-	-	-	
Block	11	24667.08	2242.46	1.39	2.82	NS
Formulation	1	11936.64	11936.64	7.39	4.84	S
Error	11	17766.56	1615.14	-	-	

Table 21. The time to peak plasma concentrations (t_{max}) of ketoprofen of twelve rabbits after rectal administration of two formulations of 100 mg ketoprofen and intramuscular administration of 50 mg Oruvail®

Rabbit No.	t_{max} (hr)		
	20% Tween 80®*	20% DMI*	Oruvail®**
1	1	0.5	0.5
2	0.5	1	1
3	0.5	0.5	0.5
4	0.5	1	1
5	0.5	1	1
6	1	1	0.5
7	1.5	1	0.5
8	0.5	1	1
9	0.5	0.5	1
10	1	1	1
11	1	1	1
12	1	0.5	1
Mean	0.79	0.83	0.83
S.D.	0.33	0.25	0.25

* 100 mg

** 50 mg

Table 22. Analysis of variance for t_{max} after rectal administration of two formulations of 100 mg ketoprofen (α=0.05)

Source of variation	d.f.	SS	MS	F ratio	F table	Sig.level
Total	23	1.91	-	-	-	
Block	11	1.03	0.09	1.19	2.82	NS
Formulation	1	0.01	0.01	0.13	4.84	NS
Error	11	0.86	0.08	-	-	

Area under the plasma ketoprofen concentration-time curve (AUC)

This parameter represents the extent or total amount of ketoprofen absorption into the systemic circulation and becomes available at the site of action. The AUC (Mean ± S.D.) from coated rectal capsule with 20% Tween 80®, 20% DMI and intramuscular injection formulation (Oruvail®) were 917.814 ± 173.044, 778.639 ± 360.958 and 361.959 ± 99.844 μg.hr/mL (Table 23), respectively. Statistical comparison in Table 24 showed that the AUC of both test formulations were not statistically significant differences ($p>0.05$), referring that the drug from the two formulations were equal in the extent of drug absorption.

Factor affecting this equal might be due to ketoprofen in the formulation with 20% Tween 80® and that with 20% DMI were already formed in clear solution. Additionally, ketoprofen, a lipophilic drug is classified as a drug with high permeability (US FDA, 1999) referring absorption process of this drug is not rate-limiting step. Absorption of the drug was taken place immediately and steadily from site of administration without any lag times providing equal amount of the drug in the systemic circulation. The extent of drug absorption in this finding based on the dose given were approximately 50% of those reported by Barba et al. This was probably due to differences in the formulation, amount of subjects or renal function of subjects. The major pathway of ketoprofen elimination was formation and subsequent urinary excretion of acyl-glucoronide conjugates and these ester conjugates were unstable and might readily hydrolyse to release parent drug. The excretion of conjugates was closely tied to renal function (Jamali and Dion, 1990; Kenneth et al., 1993)

Elimination half – life ($t_{1/2}$)

The average (Mean ± S.D.) elimination half-lives of coated rectal capsule with 20% Tween 80®, 20% DMI and from Oruvail® were 2.999 ± 0.894, 2.960 ± 1.050 and 3.321 ± 1.747 hour, respectively (Table 25). Statistical comparison in Table 26

Table 23. Area under the plasma concentration time curve (AUC) of ketoprofen of twelve rabbits after rectal administration of two formulations of 100 mg ketoprofen and intramuscular administration of 50 mg Oruvail®

Rabbit No.	AUC (μg.hr/mL)		
	20% Tween 80®*	20% DMI*	Oruvail®**
1	862.423	714.926	458.466
2	497.239	589.271	350.833
3	1002.493	329.941	419.065
4	1203.456	1551.953	249.900
5	1032.554	836.051	384.111
6	806.716	716.986	505.742
7	1022.171	959.160	270.412
8	987.608	1236.630	488.608
9	1003.024	322.805	316.013
10	820.305	957.005	427.755
11	867.376	645.576	216.340
12	908.399	483.368	256.265
Mean	917.814	778.639	361.959
S.D.	173.044	360.958	99.844

* 100 mg

** 50 mg

Table 24. Analysis of variance for AUC after rectal administration of two formulations of 100 mg ketoprofen (α=0.05)

Source of variation	d.f.	SS	MS	F ratio	F table	Sig.level
Total	23	1878794.76	-	-	-	
Block	11	1164855.01	105895.91	1.95	2.82	NS
Formulation	1	116216.47	116216.47	2.14	4.84	NS
Error	11	597723.28	54338.48	-	-	

Table 25. Elimination half-lives ($t_{1/2}$) of ketoprofen of twelve rabbits after rectal administration of two formulations of 100 mg ketoprofen and intramuscular administration of 50 mg Oruvail®

Rabbit No.	$t_{1/2}$ (hr)		
	20% Tween80®*	20 %DMI*	Oruvail®**
1	2.566	3.125	5.809
2	2.425	2.046	1.507
3	3.932	3.327	5.422
4	4.923	4.888	1.295
5	3.927	2.623	3.608
6	2.618	2.682	4.625
7	2.246	2.421	1.292
8	2.438	5.153	5.820
9	3.352	2.723	3.354
10	2.603	2.279	2.151
11	1.776	1.797	1.912
12	3.187	2.456	3.050
Mean	2.999	2.960	3.321
S.D.	0.894	1.050	1.747

* 100 mg

** 50 mg

Table 26. Analysis of variance for $t_{1/2}$ after rectal administration of two formulations of 100 mg ketoprofen (α=0.05)

Source of variation	d.f.	SS	MS	F ratio	F table	Sig.level
Total	23	20.94	-	-	-	
Block	11	15.45	1.40	2.80	2.82	NS
Formulation	1	0.01	0.01	0.02	4.84	NS
Error	11	5.48	0.50	-	-	

indicated that the $t_{1/2}$ of the two test formulations were not statistically significant differences ($p>0.05$).

All estimated pharmacokinetic parameters of ketoprofen from twelve rabbits after rectal administration of two formulations of coated ketoprofen rectal capsule as well as its relative bioavailability were summarized and shown in Table 27.

3.2 Evaluation of relative bioavailability

As mentioned earlier, bioequivalence between each of the test formulations relative to the intramuscular injection formulation (Oruvail®) could not be assessed due to unequal doses among these formulations were given. Thus, relative bioavailability was established instead. This was accomplished by comparing the area under the curve normalized by the dose administered. Results indicated that in comparison with intramuscular administration, the relative bioavailabilities of ketoprofen following rectal administration of formulation with 20% Tween 80® and that with 20% DMI were remarkably found to be 127 and 107%, respectively. It was surprised that the bioavailability of ketoprofen from both rectal formulations were superior to that from intramuscular injection formulation. This was probably due to the absorbed ketoprofen from rectal capsules bypass the liver.

Although the bioequivalence of individual coated ketoprofen rectal capsule relative to the intramuscular ketoprofen injection could not be established as anticipated, results of this study, was still useful in terms of

1. An alternative dosage form of ketoprofen in addition to oral and intramuscular injection formulations was accomplished.

2. The extent of drug absorption from these alternatives were superior to that from the intramuscular injection form.

However, further studies in human subjects are needed in order to summarize final conclusion regarding to its availability.

Table 27. Estimated pharmacokinetic parameters of ketoprofen (Mean ±S.D.) from twelve rabbits after rectal administration of formulation with 20% Tween 80® coated rectal capsule and formulation of 20% DMI coated rectal capsule and its relative bioavailability

Pharmacokinetic parameters	Formulations		Statistical Test
	20% Tween 80®	20% DMI	
C_{max} (µg / mL)	238.562 ± 37.163	193.958 ± 49.764	S
AUC (µg.hr / mL)	917.814 ± 173.044	778.639 ± 360.958	NS
t_{max} (hr)	0.790 ± 0.330	0.830 ± 0.250	NS
$t_{1/2}$ (hr)	2.999 ± 0.894	2.960 ± 1.050	NS
F_{rel} (%)	126.78	107.56	-