CHAPTER II

MATERIALS AND METHODS

A. Materials

1. Drugs

- Prednisolone USP/BP, anhydrous micronized (Lot No. 305 HK, The Upjohn Company, U.S.A.)
- Indomethacin, BP 73 (Lot No. 791001, Pharmaceutical Science Ltd., Part., Bangkok, Thailand.)

2. Carriers

- Dextrose anhydrous USP/BP (Batch No. 2218 A1, Iwaki, Japan.)
- Polyethylene glycol 4000 (Pharmaceutical Science Ltd., Part., Bangkok, Thailand.)
- Sodium lauryl sulfate (Pharmaceutical Science Ltd., Part., Bangkok, Thailand.)

3. Additives

- Dicalcium phosphate dihydrate (Lot No. 9980, Pharmaceutical Science Ltd., Part., Bangkok, Thailand.)
- Microcrystalline cellulose (Avicel PH 102, Lot No. 2743, AMC Corporation Ltd., Bangkok, Thailand.)
- Cab-o-sil (Pharmaceutical Science Ltd., Part., Bangkok, Thailand.)

4. Others

- Absolute ethanol (Lot No. K3813783, Merck, Germany.)
- Potassium dihydrogen phosphate (Lot No. 240434, Fluka-Garantie, Switzerland.)
- Sodium hydroxide (Lot. No. 8070408, Eka Kemi, Sweden.)

B. Equipment

- Analytical balance (Sartorius, Model 2442, Germany.)
- Mortar and Pestle
- Sieve No. 40 (Endecotts Ltd., England.)
- Hardness Tester (Schleuniger, Model 2E/205, Dr. K. Schleuniger Co., Switzerland.)
- Single Punch Tablet Machine (Viuhang Engineering, No. 6, Thailand.)
- Disintegration apparatus (Manesty T.D. 65T 170, Manesty Machines Ltd., England.)
- Dissolution apparatus U.S.P. type I (Hanson Research Corporation, Model 500-230, Northridge, California, U.S.A.)
- pH meter (Pye Model 292 pH meter, Pye Unicam Ltd., Cambridge, England.)
- Spectrophotometer (Beckman, Model 3600 Spectrophotometer, U.S.A.)

C. Methods

1. Preparation of Prednisolone or Indomethacin Coprecipitates

Prednisolone or indomethacin coprecipitates were prepared by dissolving weighed quantities of prednisolone or indomethacin and carrier(s), presented in Table 2 and 3, in 30 or 60 ml absolute ethanol respectively. In the case where dextrose or SLS was employed as carrier, dextrose or SLS was dissolved first in adequate amount of distilled water. Then, the aqueous solution of dextrose or SLS was incorporated into ethanolic solution of drug or drug and PEG 4000.

The solutions were mixed thoroughly and the solvent was allowed to evaporate continuously under a cold air stream. The resulting coprecipitates were placed in an incubator at 50°C overnight to eliminate the remaining solvent and then were stored in a dessicator until the dry solid coprecipitates were obtained. The dry coprecipitates then were screened through a 40 mesh sieve and kept in a dessicator.

2. Preparation of Prednisolone or Indomethacin physical mixtures

The required amounts of prednisolone or indomethacin and carrier(s), presented in Table 2 and 3, were thoroughly mixed using a mortar and pestle for 5 minutes. The mixture was sieved through a 40 mesh sieve and stored in a dessicator.

Table 2. Quantities of Prednisolone and Carrier(s) Used in Preparing Coprecipitates or Physical Mixtures

COPRECIPITATE OR PHYSICAL MIXTURE	PREDNISOLONE (g)	DEXTROSE (g)	PEG 4000 (g)	SLS (g)
1:0 Prednisolone:carrier	0.5			
1:1 Prednisolone:dextrose	0.5	0.5		
1:3 Prednisolone:dextrose	0.5	1.5		
1:5 Prednisolone:dextrose	0.5	2.5		
1:1 Prednisolone:PEG 4000	0.5		0.5	
1:3 Prednisolone:PEG 4000	0.5		1.5	
1:5 Prednisolone:PEG 4000	0.5		2.5	
1:1 Prednisolone:SLS	0.5			0.5
1:3 Prednisolone:SLS	0.5			1.5
1:5 Prednisolone:SLS	0.5			2.5
1:(1+4) Prednisolone:(dextrose+PEG 4000)	0.5	0.5	2.0	
1:(2+3) Prednisolone:(dextrose+PEG 4000)	0.5	1.0	1.5	
1:(3+2) Prednisolone:(dextrose+PEG 4000)	0.5	1.5	1.0	
1:(4+1) Prednisolone:(dextrose+PEG 4000)	0.5	2.0	0.5	
1:(1+4) Prednisolone:(dextrose+SLS)	0.5	0.5		2.0
1:(2+3) Prednisolone:(dextrose+SLS)	0.5	1.0		1.5
1:(3+2) Prednisolone:(dextrose+SLS)	0.5	1.5		1.0
1:(4+1) Prednisolone:(dextrose+SLS)	0.5	2.0		0.5

Table 3. Quantities of Indomethacin and Carrier(s) Used in Preparing Coprecipitates and Physical Mixtures

COPRECIPITATE OR PHYSICAL MIXTURE	INDOMETHACIN (g)	DEXTROSE (g)	PEG 4000 (g)	SLS (g)
1:0 Indomethacin:carrier	2.5			
1:1 Indomethacin:dextrose	2.5	2.5		
1:1 Indomethacin:PEG 4000	2.5		2.5	
1:1 Indomethacin:SLS	2.5			2.5
1:(0.2+0.8) Indomethacin:(dextrose+PEG 4000)	2.5	0.5	2.0	
1:(0.4+0.6) Indomethacin:(dextrose+PEG 4000)	2.5	1.0	1.5	
1:(0.6+0.4) Indomethacin:(dextrose+PEG 4000)	2.5	1.5	1.0	
1:(0.8+0.2) Indomethacin:(dextrose+PEG 4000)	2.5	2.0	0.5	
1:(0.2+0.8) Indomethacin:(dextrose+SLS)	2.5	0.5		2.0
1:(0.4+0.6) Indomethacin:(dextrose+SLS)	2.5	1.0		1.5
1:(0.6+0.4) Indomethacin:(dextrose+SLS)	2.5	1.5		1.0
1:(0.8+0.2) Indomethacin:(dextrose+SLS)	2.5	2.0		0.5

3. Preparation of Test Tablets

The prednisolone or indomethacin copreciptiates or the prednisolone or indomethacin physical mixtures were incorporated into direct compression tablets by the following procedure.

The required quantity of coprecipitate or physical mixture was mixed with the required quantity of dicalcium phosphate dihydrate using a mortar and pestle by geometric dilution for 3 minutes. Avicel PH 102 and cab-o-sil were then added to the mixture and were mixed together for 5 minutes in the mortar. The obtained mixture was passed through a 40 mesh sieve and was directly compressed into tablets using a single punch tablet machine. The tablets were prepared to have a diameter of 9 mm, and a hardness between 4-5 Kp.

The composition of each formula was described in Table 4 and 5. The prepared tablets were kept in glass containers and stored in a dessicator for the further studies.

Table 4. Composition of Prednisolone Test Tablets

FORMULA	PREDNISOLONE (mg)	DEXTROSE (mg)	PREDNISOLONE:DEXTROSE COPRECIPITATE (mg)	AVICEL PH 102 (mg)	DICALCIUM PHOSPHATE (mg)	CAB-O-SIL (mg)
1*	5			50	145	6
2	5	5		50	145	6
3	5	15		50	145	6
4	5	25		50	145	6
5			5:5	50	145	6
6			5:15	50	145	6
7			5:25	50	145	6

* Prednisolone control tablets containing prednisolone recrystallized from absolute ethanol

Table 4. (Continued) Composition of Prednisolone Test Tablets

FORMULA	PREDNISOLONE (mg)	PEG 4000 (mg)	SLS (mg)	PREDNISOLONE:PEG 4000 COPRECIPITATE (mg)	AVICEL PH 102 (mg)	DICALCIUM PHOSPHATE (mg)	CAB-O-SIL (mg)
8	5	5			50	145	6
9	5	15			50	145	6
10	5	25			50	145	6
11				5:5	50	145	6
12				5:15	50	145	6
13				5:25	50	145	6
				PREDNISOLONE:SLS COPRECIPITATE (mg)			
14	5		5		50	145	6
15	5		15		50	145	6
16	5		25		50	145	6
17				5:5	50	145	6
18				5:15	50	145	6
19				5:25	50	145	6

Table 4. (Continued) Composition of Prednisolone Test Tablets

FORMULA	PREDNISOLONE (mg)	DEXTROSE (mg)	PEG 4000 (mg)	SLS (mg)	PREDNISOLONE: (DEXTROSE+PEG 4000) COPRECIPITATE (mg)	AVICEL PH 102 (mg)	DICALCIUM PHOSPHATE (mg)	CAB-O-SIL (mg)
20	5	5	20			50	145	6
21	5	10	15			50	145	6
22	5	15	10			50	145	6
23	5	20	5			50	145	6
24					5:(5+20)	50	145	6
25					5:(10+15)	50	145	6
26					5:(15+10)	50	145	6
27					5:(20+ 5)	50	145	6
					PREDNISOLONE: (DEXTROSE+SLS) COPRECIPITATE (mg)			
28	5	5		20		50	145	6
29	5	10		15		50	145	6
30	5	15		10		50	145	6
31	5	20		5		50	145	6
32					5:(5+20)	50	145	6
33					5:(10+15)	50	145	6
34					5:(15+10)	50	145	6
35					5:(20+ 5)	50	145	6

Table 5. Composition of Indomethacin Test Tablets

FORMULA	INDOMETHACIN (mg)	DEXTROSE (mg)	PEG 4000 (mg)	SLS (mg)	INDOMETHACIN :DEXTROSE COPRECIPITATE (mg)	INDOMETHACIN :PEG 4000 COPRECIPITATE (mg)	INDOMETHACIN :SLS COPRECIPITATE (mg)	AVICEL PH 102 (mg)	DICALCIUM PHOSPHATE (mg)	CAB-O-SIL (mg)
36*	25							50	145	6
37	25	25						50	145	6
38					25:25			50	145	6
39	25		25					50	145	6
40						25:25		50	145	6
41	25			25				50	145	6
42							25:25	50	145	6

* Indomethacin control tablets containing indomethacin recrystallized from absolute ethanol

Table 5. (Continued) Composition of Indomethacin Test Tablets

FORMULA	INDOMETHACIN (mg)	DEXTROSE (mg)	PEG 4000 (mg)	SLS (mg)	INDOMETHACIN: (DEXTROSE+PEG 4000) (mg)	AVICEL PH 102 (mg)	DICALCIUM PHOSPHATE (mg)	CAB-O-SIL (mg)
43	25	5	20			50	145	6
44	25	10	15			50	145	6
45	25	15	10			50	145	6
46	25	20	5			50	145	6
47					25:(5+20)	50	145	6
48					25:(10+15)	50	145	6
49					25:(15+10)	50	145	6
50					25:(20+ 5)	50	145	6
					INDOMETHACIN: (DEXTROSE+SLS) (mg)			
51	25	5		20		50	145	6
52	25	10		15		50	145	6
53	25	15		10		50	145	6
54	25	20		5		50	145	6
55					25:(5+20)	50	145	6
56					25:(10+15)	50	145	6
57					25:(15+10)	50	145	6
58					25:(20+ 5)	50	145	6

D. In-vitro Studies

1. Disintegration Studies

Disintegration time studies were conducted on test tablets using USP XX disintegration apparatus. The average disintegration time was obtained from 6 tablets. The temperature of 37±1 °C was maintained throughout the experiment. Water was used as disintegration medium.

2. Dissolution Studies

2.1 Dissolution Rate Studies of Prednisolone tablets

Dissolution rate studies of prednisolone tablets were performed according to USP XX (7). Nine hundred millilitres of distilled water was used as dissolution medium. The dissolution medium was maintained at 37±1 °C. The basket was adjusted to rotate at 100 rpm. Ten millilitre aliquots of the dissolution medium were pipetted out at the interval of 1, 3, 5, 7, 10, 15, 20, 30, 45, and 60 minutes, and then were filtered using filter paper. About 5 ml of the first filtrate was discarded and the subsequent filtrate was used as the sample solution. The volume withdrawn at each time interval was replaced with fresh quantity of dissolution medium.

Assay- The sample solutions were assayed spectrophotometrically at 246 nm for prednisolone content. The sample concentrations were calculated from standard curve.

Standard curve- Standard curve of prednisolone in dissolution medium was obtained between 2-10 mcg/ml. Absorbance at 246 nm versus

concentration plots, as presented in Figure 2, revealed that Beer's law was followed.

2.2 Dissolution Rate Studies of Indomethacin Tablets

Dissolution rate studies of indomethacin tablets were performed according to the dissolution of indomethacin capsules, USP XXI & NF XVI (2). Seven hundred and fifty millilitres of a mixture of distilled water and pH 7.2 phosphate buffer solution (4:1) was used as dissolution medium. The following indomethacin dissolution studies were the same as described in dissolution studies for prednisolone tablets.

Assay- The sample solutions were assayed spectrophotometrically at 318 nm for indomethacin content. The sample concentrations were calculated from standard curve.

Standard curve- Standard curve of indomethacin in dissolution medium was obtained between 10-50 mcg/ml. Absorbance at 318 nm versus concentration plots, as demonstrated in Figure 3, followed Beer's law.

Note: Dissolution rate studies of prednisolone and indomethacin were performed in duplicate.

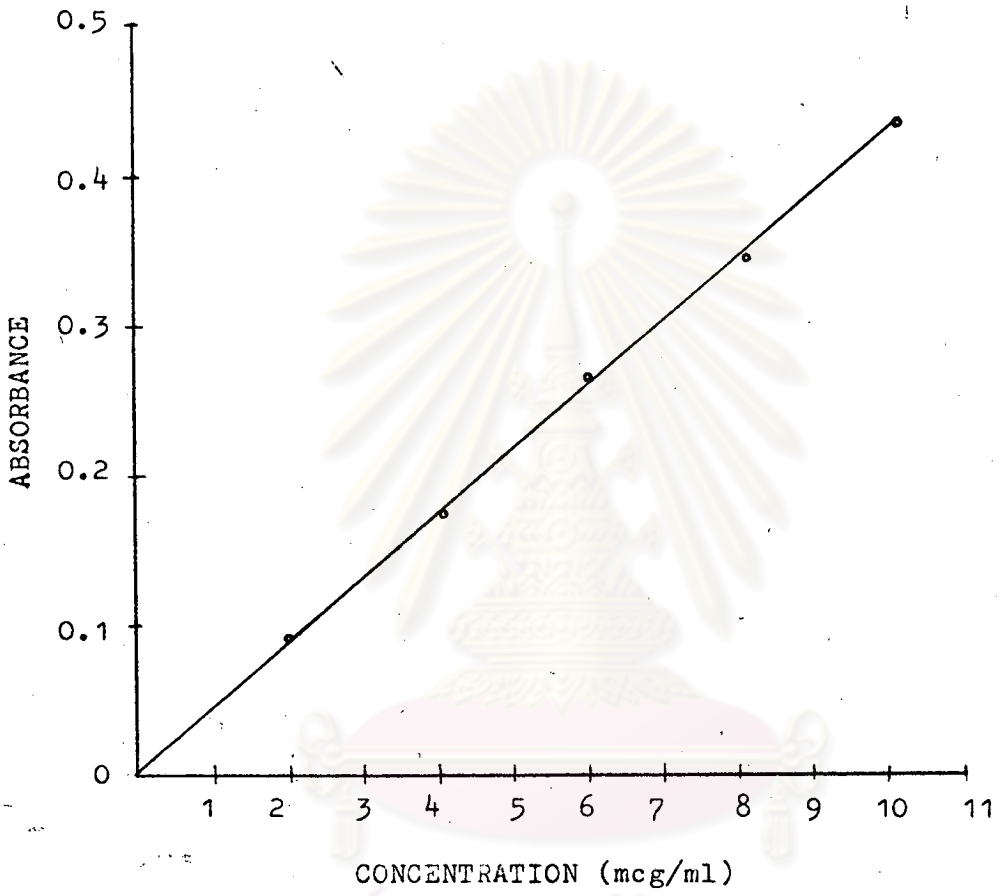

Figure 2. Standard curve plotting the concentration of prednisolone versus absorbance at 246 nm.
Slope = 0.042 ml/mcg. (n =2)

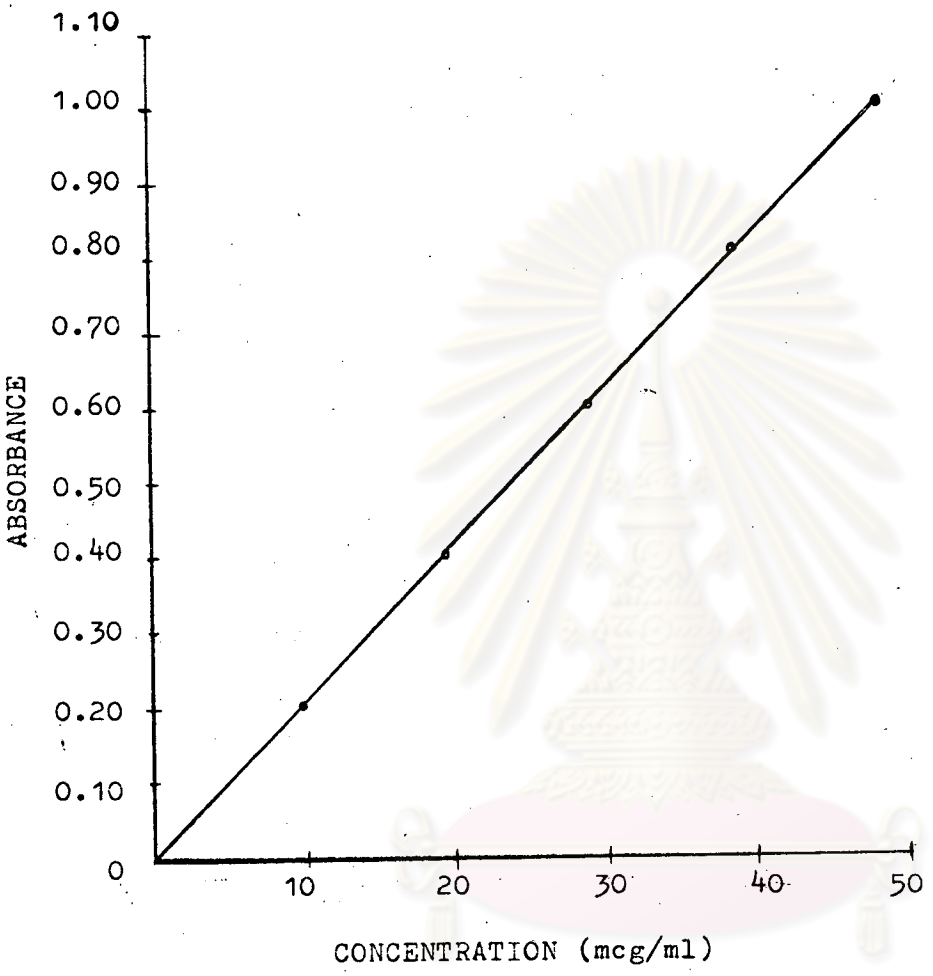

Figure 3. Standard curve plotting the concentration of indomethacin versus absorbance at 318 nm. Slope = 0.021 ml/mcg. (n = 2)