

Chapter II

Materials and Methods

Materials

The following materials were purchased from commercial sources. Purified water was used through out this study.

1. Model drug

Ibuprofen B.P. (Sekhsaria Chemicals Limited, India, Batch No.9302233)

Calcium Carbonate (Korea)

2. Starch materials

Corn starch (Bestfood, Thailand)

Glutinous rice starch (NEWGRADE, Thai Wah Food Products, Thailand)

Rice starch (Bai Yok, Bangkok Inter Food, Thailand)

Tapioca starch (Food Grade, Thai Wah Co.Ltd., Thailand)

3. Commercial Suspending agents

Acacia (Brampton Co. J.W., U.S.A.)

Avicel RC-591 (Lot No. B 421, FMC, U.S.A.)

Sodium alginate (China)

Sodium Carboxymethylcellulose (PMC, U.S.A.)

Tragacanth (Lot No. 88.292, Merck, Germany)

Xanthan gum (supplied by RAMA, Thailand)

4. Material used for modification of starch

Acetic acid (Lot No. 7313, Riedel-de Haen, Germany)

Chloroacetic acid (Lot No. 111H0240, Sigma, U.S.A.)

Ethanol absolute (Lot No. K18464283, Merck, Germany)

Glacial acetic acid (Lot No. K18049863, Merck, Germany)

Isopropanol absolute (Lot No. V5C550095C, Carlo Erba, Italy)

Methanol absolute (Lot No. 3041KPDE, Mallinckrodt, U.S.A.)

Sodium hydroxide (J.T.Baker, U.S.A.)

5. Other suspension additives

Methyl paraben (Lot No. MFB16, Japan)

Propyl paraben (Lot No. UB19/1, Japan)

Sodium saccharin (Fisher, U.S.A.)

Sorbitol (Lot No. 865186, Fisher Biotech, U.S.A.)

Sucrose (Mitrphol, Thailand)

Tween^R 80 (Lot No. 882623, Fisher Biotech, U.S.A.)

6. Others

Sulfuric acid (Lot No. 733696, Fisher Scientific Co., U.S.A.)

Apparatus

Analytical balance (Sartorius, Germany)

Hot air oven (Mettler, type ULSO, Germany)

Fourier-Transformed Infrared Spectrophotometer (Perkin Elmer, 1760X,
U.S.A.)

Mechanical sieve shaker (Josef Deckelmann, Aschattenberg, Western)

Magnetic stirrer (SP-18420, Nuova 7 Stir-Plate, Sybron Thermolyne, U.S.A.)

Rheometer (Haake Viscometer, Model Rotovisco RV 20, West Germany)

Ultraviolet Spectrophotometer (Milton Roy Spectronic, Model 3000 Array,
Milton Roy Company, U.S.A.)

Methods

A. Preparation and Determination of Modified Starches

1. Preparation of Modified Starches

Modified starches were prepared from four native starch powders, including rice starch, glutinous rice starch, corn starch, and tapioca starch, by carboxymethyl substitution using the method modified from that described by Filbert (1952). Monochloroacetic acid (MCA) was used as a reagent, under basic condition, in the reaction. To obtain three different degrees of substitution, the following methods were used.

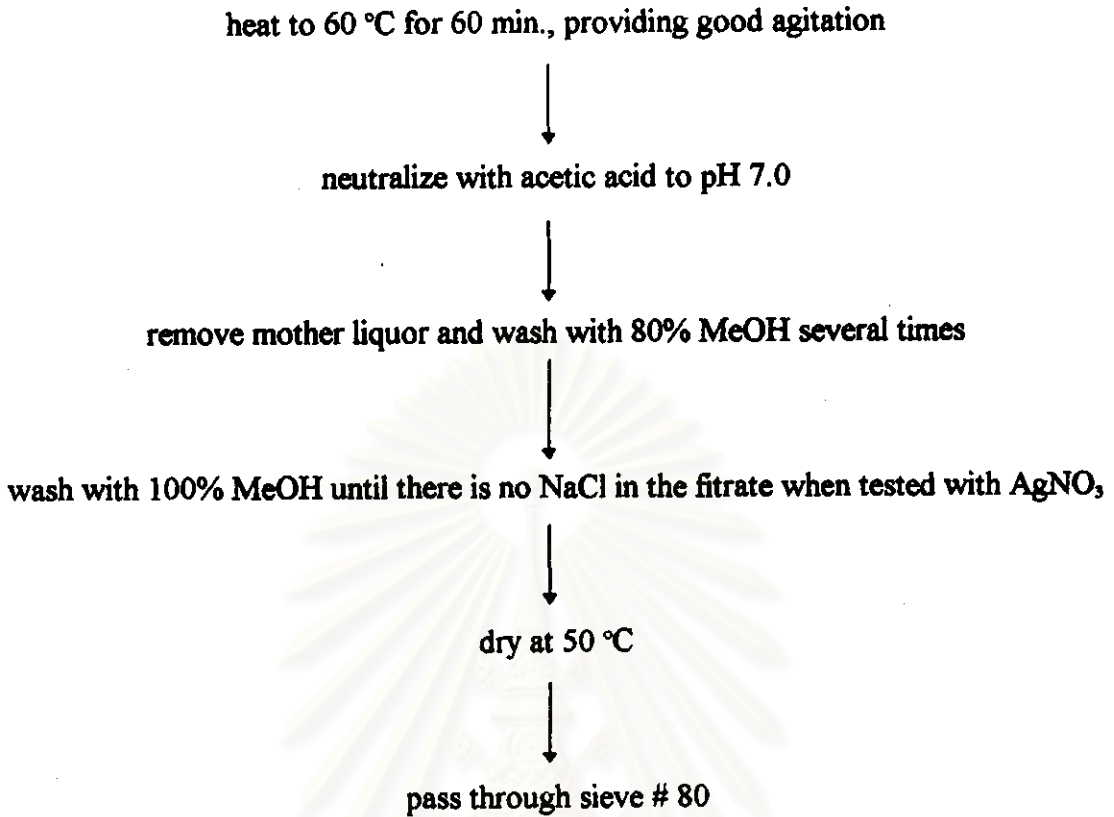
1.1 Degree of substitution of 0.13

254 g of 100% MeOH + 27.6 g of monochloroacetic acid (MCA)

↓
add 109 g of starch

↓
add 110 g of 50% aqueous solution NaOH

↓



1.2 Degree of substitution of 0.26

286 g of EtOH (92.4% w/w), 29.2 g of MCA, and 102 g of starch were mixed

to form slurry

add 38.4 g of flake 97% NaOH dissolved in 69.0 g of water with maintainance of

slurry temperature below 50 °C

agitation at 50 °C for 20 minutes

neutralized with acetic acid

remove mother liquor and wash with 80% MeOH several times

wash with 100% MeOH until there is no NaCl in the filtrate when tested with AgNO₃

dry at 50 °C

pass through sieve # 80

1.3 Degree of substitution of 0.39

286 g of EtOH (92.4% w/w), 29.2 g of MCA, and 102 parts of starch were mixed

to form slurry

add 38.4 g of flake 97% NaOH dissolved in 69.0 g of water with maintainance of

slurry temperature below 50 °C

agitation at 50 °C for 2 hours

neutralized with acetic acid

remove mother liquor and wash with 80% MeOH several times

wash with 100% MeOH until there is no NaCl in the filtrate when tested with AgNO₃

dry at 50 °C
↓
pass through sieve # 80

2. Determination of a Degree of Substitution (D.S.)

The determination of a degree of substitution (DS) was performed by the method for Croscarmellose Sodium as described in USP XXIII. The procedures include titration step and residue on ignition step. From these steps, the number of milliequivalents of base required for the neutralization of 1 g sample (*M*) and the percentage of Residue on Ignition (*C*) were obtained. Each sample was tested in triplicated. The procedures are as followed;

Titration Step

Transfer about 1 g of modified starch, accurately weighed,
to a glass-stoppered, 500 ml conical flask

↓
add 300 ml of NaCl solution (1 in 10)

↓
add 25.0 ml of 0.1 N NaOH

↓
insert the stopper, and allow to stand for five minutes with intermittent shaking

↓

add 5 drops of m-cresol purple TS and about 15 ml of 0.1 N HCl from a buret



insert the stopper in the flask and shake, if the solution is purple add 0.1 N HCl in 1 ml portions until the solution becomes yellow, shaking after each addition



titrate with 0.1 N NaOH to a purple endpoint



calculate the net number of milliequivalents, M , of base required for the neutralization of 1 g of modified starch, on the dried basis



determine the percentage of residue on ignition, C , of modified starch on the dried basis as described under Residue on Ignition Step

Residue on Ignition Step

weigh accurately 1 g of substance in a suitable crucible

(The crucible has previously been ignited, cooled and weighed)



heat, gently at first, until the substance is thoroughly charred, cool



moisten the residue with 1 ml of H_2SO_4



heat gently until white fumes are no longer evolved



ignited at 800 ± 25 °C until the carbon is consumed



cool in a desiccator, weigh, and calculate the percentage of residue

The degree of substitution (D.S.) can be calculated by the formula

$$D.S. = A + S$$

A = degree of acid carboxymethyl substitution

$$= 1150M/(7102-412M-80C)$$

S = degree of sodium carboxymethyl substitution

$$= (162+58A)C/(7102-80C)$$

3. Detection of Carboxymethyl Group in Substituted Starches

The presence of carboxymethyl group in substituted starches was confirmed by the detection of carbonyl group with Fourier-Transformed Infrared spectrophotometer (FT-IR). The samples were prepared as KBr pellets and were scanned four times with the speed of three seconds per scan. The resolution was four. The comparison was made between IR spectrum of native starch and IR spectrum of modified starch.

B. Evaluation of Modified Starches as Suspending Agent

1. Viscosity Determination of Modified Starches

The viscosities of four modified starches at concentration 1, 2, and 3% w/v were determined by Rotovisco RV20 rheometer (HAAKE Mess-Technik GmbH u. Co.). The measurements were performed at 25.8 °C in all experiments. The starches

were first hydrated overnight to allow complete swelling. The fully hydrated starch samples were then loaded and the shear rate was increased from 0 s^{-1} to 1000 s^{-1} in one minute. The shear rate was maintained at 1000 s^{-1} for three minutes and a total number of ten values of shear stress were observed and recorded consistently. The experiments were repeated twice. The values obtained from these observations were averaged and were used to calculate the viscosity of sample by the following equation:

$$\text{Viscosity} = \frac{\text{Shear Stress (Pa)}}{\text{Shear Rate (s}^{-1}\text{)}}$$

2. Application of Modified Starches in Calcium Carbonate Suspension

2.1 Preparation of Calcium Carbonate Suspension

The 10% w/v calcium carbonate suspensions were prepared with varied concentration of modified starches (1%, 2%, 3% w/v). The formulation is as followed:

Calcium Carbonate	10%
Methyl Paraben (MP)	0.18%
Propyl Paraben (PP)	0.02%
Modified Starches	1% or 2% or 3%
Purified Water qs ad	100 mL

The preparation method is as followed:

dissolve methyl paraben and propyl paraben in water



hydrate modified starch in water that contained MP and PP



levigate CaCO₃ in mortar with modified starch paste



adjust volume with water and mix thoroughly



pass through hand homogenizer



deflocculated suspension

2.2 Determination of Sedimentation Volume of CaCO₃ Suspension

The sedimentation volume of CaCO₃ suspension was determined at room temperature (about 25 °C) over a period of one month using the cylindrical graduate method (Nasipuri and Ogumlana, 1978). The procedure is as follows: 50 mL of triplicate were stored in 100 mL graduated glass cylinders. Each sample was shaken to ensure uniformity prior to the study. Sedimentation height was measured and recorded every two days without disturbing the suspension. The sedimentation volume (F) was calculated from the ratio of the ultimate height (Hu) of the sediment to the initial height (Ho) of the total suspension.

$$\text{Sedimentation Volume (SV)} = \frac{H_u}{H_o}$$

2.3 Determination of Redispersibility of Calcium Carbonate Suspension

The ease of redispersion was deduced from the number of the times each test tube had to be inverted, by hand, under reasonably-controlled conditions, before the sediment was completely resuspended (Farley and Lund, 1976). In this study, the triplicated 10 mL test tubes that were allowed to settle for 1, 2, 3, and 4 weeks were tested. Based on the number of inversion and the effort required to obtain homogeneous suspension, the formulations were evaluated. If complete redispersion was not achieved after 12 inversions of the test tube, it was shaken vigorously. If after shaking vigorously the deposit was still present the system was described as "caked".

C. Rheological Studies of Pure Suspending Agents

1. Rheological Determination of Selected Modified Starches

After the preliminary evaluation of modified starches with different degrees of substitution, a modified starch that exhibit the best properties as suspending agent from each group was selected. The rheological behaviors of these modified starches were subsequently evaluated in comparison with other commercially available suspending agents. These include xanthan gum (XG), sodium alginate (SA), Avicel[®] RC-591(AV), tragacanth (TG), acacia (AC), and sodium carboxymethylcellulose (SCMC). The rheological behaviors of these suspending agents were studied at the concentration of 1, 2, or 3% by Haake viscometer at 25.8 °C. The measuring system was M5. For the highly viscous compound (i.e. xanthan gum), a SV1 sensor was used while the MV1 and NV sensors were used for intermediate viscous compounds (i.e. sodium alginate, tragacanth, MGS, MTS, MRS, SCMC) and low viscous compounds (i.e. acacia,

Avicel), respectively. The shear rate used was 1000 s^{-1} . One cycle consisted of two minutes to allow the shear rate to reach $1,000 \text{ s}^{-1}$, then maintained at this value for four minutes. The last part was two minutes to decrease the shear rate back to 0 s^{-1} . The complete cycle takes eight minutes. Shear stress values from 20 different points of triplicate observation were used to plot a rheology curve of each suspending agent.

2. Measurement of Thixotropy

Thixotropic values of each suspending agents were determined as a value of thixotropic breakdown. This was calculated from the area of a hysteresis loop formed between the upcurve and downcurve by the equation;

$$\text{Thixotropic Breakdown} = \text{Area Under Upcurve} - \text{Area Under Downcurve}$$

D. Application of Selected Modified Starches in Ibuprofen Suspension Formulation

1. Preparation of Ibuprofen Suspension

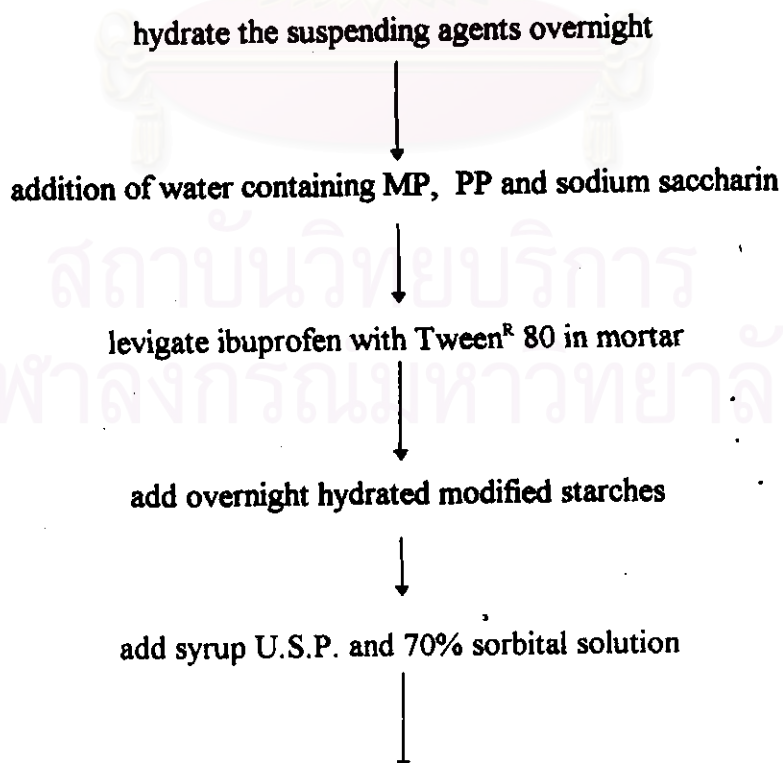
Ibuprofen suspensions (100 mg/5 mL) were prepared by the following formulation.

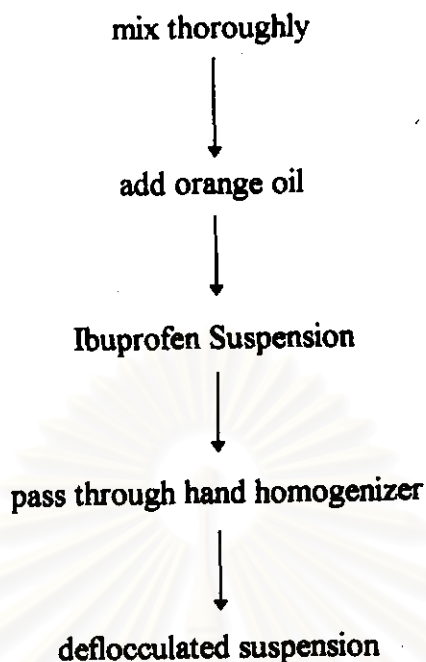
Ibuprofen	2	g
Tween 80	0.25	g
Suspending Agent*	1,2,3	g
Syrup USP	15	ml
70 % Sorbitol Solution	15	ml

Sodium Saccharin	0.02	g
Methyl Paraben (MP)	0.18	g
Propyl Paraben(PP)	0.02	g
Orange Oil	10	drops
2 % Sunset Yellow	5	drops
Purified Water qs ad	100	mL

* Suspending agents used for comparative studies were Xanthan gum (XG), Sodium alginate (SA), Avicel RC-591 (AV), Acacia (AC), Tragacanth (TG), Sodium Carboxymethylcellulose (SCMC), Modified Rice Starch (MRS), Modified Tapioca Starch (MTS), and Modified Glutinous Rice Starch (MGS)

The preparation procedure is as follow:





2. Evaluation of the Formulation

2.1 Evaluation Condition

The evaluation of Ibuprofen suspensions was performed under two different conditions. One set of sample was evaluated under normal condition at room temperature (25 °C). The other set was evaluated under freeze-thaw cycle condition.

In the freeze-thaw cycle evaluation, the total number of eight cycles were performed at 8 °C and 45 °C. Each cycle consisted of two days storage at 8 °C and two days at 45 °C (British Pharmaceutical Codex, 1996).

2.2 Evaluation Methods and Parameters

2.2.1 Sedimentation Volume Determination of Ibuprofen Suspension

The ibuprofen suspension was evaluated by sedimentation volume determination as described. In this case, the determination was performed at suitable

time interval over a period of 12 weeks for normal condition and at the end of the test for freeze-thaw condition.

2.2.2 Redispersibility of Ibuprofen Suspension

The redispersibility of ibuprofen suspension was evaluated as the method described previously in the redispersibility determination of calcium carbonate suspension. The samples were tested after 2, 4, 6, 8, 10, and 12 weeks.

2.2.3 Rheological Studies of Ibuprofen Suspension

The rheological behaviors of freshly prepared ibuprofen suspensions, 12 weeks old ibuprofen suspensions, and freeze-thaw cycle treatment ibuprofen suspensions containing different suspending agents were investigated by plotting the flow curves, comparing the viscosity, as well as determining the thixotropic quantity. The conditions of measurement were the same as mentioned previously in the determination of rheological behaviors of pure modified starches.

2.2.4 Study of Uniformity of Drug Dispersion During Storage

2.2.4.1 Preparation of Standard Curve

Ibuprofen 50 mg was accurately weighed and dissolved in phosphate buffer pH 7.2 (USP XXIII, see Appendix IV). The solution was adjusted to 50 mL with phosphate buffer pH 7.2 to obtain a stock solution (1 mg/mL). The stock solution was individually pipetted 0.2, 0.6, 1.0, 1.4, and 1.8 mL, transferred into five 50 mL volumetric flasks, and diluted to volume with phosphate buffer pH 7.2. The final

concentration of each solution was 0.4, 1.2, 2.0, 2.8, and 3.6 mg/100 mL, respectively.

The absorbance of each standard solution was determined using UV spectrophotometer in a 1 cm cell at 223 nm (see Ibuprofen Spectra Scan for λ_{max} in Figure 83, Appendix I). The phosphate buffer pH 7.2 was used as a blank solution. Each concentration was determined in triplicated.

2.2.4.2 Determination of Uniformity of drug dispersion during storage of Ibuprofen suspension

The uniformity of drug dispersion during storage of ibuprofen was studied on selected suspensions. The suspensions were selected from those that showed good result in sedimentation volume study. The procedures are as follow : 50 μ L aliquot of suspension sample was withdrawn from the cylinders and transferred into a 50 mL volumetric flask. The sample was dissolved, diluted, and brought up to volume with phosphate buffer pH 7.2. The absorbance of ibuprofen was measured at 223 nm by a UV spectrophotometer, using matrix solution as a blank. The measurement was performed three times by withdrawing suspension samples from three different depth levels of the cylinder. All measurements were performed at room temperature. Samples were taken and analyzed every two weeks over a period of 12 weeks. The uniformity of drug dispersion during storage was determined by the calculating the deviation of ibuprofen contents obtained from three depth levels of the cylinder over these period of time. This test was performed only on suspensions stored under normal condition.