Chapter 2

Experimental

1. Materials

All materials obtained from commercial sources were used as received.

- Indomethacin (Batch No. 850602, China)
- Pindolol (Lot No. 55F0749, Sigma, USA)
- Chitosan (MW. 30000-50000 Dalton, Distributed by G.T. Chemical Co. Ltd., Bangkok)
- Carboxymethylcellulose Sodium (Lot No. 7532C6, Distributed by Bhaesatpanit Co. Ltd., Bangkok,)
- Glutaraldehyde (Lot No. 1-83M2, Union Carbide, USA)
- Acetic acid glacial (E. Merck, Germany)
- Chloroform AR grade (BDH, England)
- Hydrochloric acid AR grade (Carlo Erba, Milano)
- Isopropanol commercial grade (Distributed by Chaimongkol Trading, Bangkok)
- Methanol AR grade (Mallinckrode, Kentucky)
- Potassium dihydrogen phosphate AR grade (E. Merck, Germany)
- Sodium hydroxide AR grade (Eka Noble, Sweden)
- Nitrogen gas (Distributed by Num Heng Co. Ltd., Bangkok)

2. Equipment

- Spray nozzle (UniGlatt Laboratory unit, Germany)
- Peristaltic pump (UniGlatt Laboratory unit, Germany)
- Air pump (serial no. 357552, USA)
- Plastic bath (9 cm diameter)
- Magnetic stirrer (Model M21/1, Framo)
- Magnetic bar (10.5 cm long)

- Analytical balance (Model A200S, Sartorius, Germany)
- pH meter (Model HI8417, Hanna Instruments, USA)
- Spectrophotometer (Spectronic 2000, Bausch & Lomb, USA)
- Dissolution apparatus (Model SR2, Hanson Research, USA)
- Photomicrograph microscope (BHB, Olympus, Japan)
- Scanning electron microscope (JSM-T220A, Jeol, Japan)
- Capsule filling machine

3. Microencapsulation Method

3.1 Preliminary Study

Chitosan solutions of varying concentration from 0.25-1.0% w/v were prepared by dispersing chitosan in acetic acid solution (1.0% w/v) and allowing it to hydrate overnight, then filtered to remove insoluble fraction. After filtration chitosan solution was adjusted to the pH 3, pH 4 and pH 5 with 1.0 N. hydrochloric acid or 1.0 N. sodium hydroxide. Finally, chitosan solution were adjusted by acetic acid solution to 800 ml volume.

Carboxymethylcellulose (CMC) solutions of 0.5, 1.0, and 2.0% w/v were prepared by dispersing CMC in deionised water and allowing it to hydrate overnight.

One hundred and twenty millilitres of CMC solution was discharged through a nozzle by peristaltic pump at 1.2 rpm. It was sprayed through the nozzle with 1.5 psi air pressure into a bath of 800 millilitres chitosan solution which had been kept at the predetermined temperature of 5, 15, and 25 ± 2°C. During the spraying the chitosan solution had to be stirred at a stirrer speed of 650 ± 50 rpm, in order to produce a vortex without air bubbles. After spraying, glutaraldehyde 0.5 gm/polymer 1 gm was added into the reaction bath, and the mixture was then stirred for 3 hours to harden the microcapsules membrane (as shown in Figure 11).

The hardened microcapsules were filtered before they were washed 3 times with water 150 millilitres in order to remove excess glutaraldehyde and 4 times with isopropanol (IPA) 80 millilitres to remove the remaining water for the microcapsules prepared from chitosan pH 4 and pH 5 solution, then they were dried with nitrogen gas. For microcapsules prepared from chitosan pH 3 solution they were washed with water 6 times and only 1 time with IPA before drying with nitrogen gas. Finally the dried microcapsules were passed through a no.#40 sieve and collected.

3.2 Microencapsulation of Pharmaceutical

From the preliminary studies of the process, the optimal temperature of chitosan solution and the concentration of CMC solution used in the preparation of pharmaceutical microcapsules were considered to be 15±2°c and 1.0% w/v respectively. The experiment conducted followed the microencapsulation process described in section 3.1, but used drug-CMC dispersion instead of CMC solution. Drug-CMC dispersion was prepared by dispersing indomethacin or pindolol, which had been passed through no.#80 sieve, in the CMC solution. The concentration of drug in CMC solution was 1.0% w/v.

The variable factors for these processes were pH of chitosan solution (pH 3, pH 4, pH 5), hardening time (1, 3, 5 hours), the amount of glutaraldehyde (0.25-2.0 gm/polymer 1 gm), and concentration of chitosan solution (0.25-1.0% w/v).

The yielded dried microcapsules were filled into capsules no. 0 by capsule filling machine without other diluent. Each capsule was contained 10 mg of pindolol or 50 mg of indomethacin. The conditions for prepared pharmaceutical microcapsules were shown in Table 4 and 5.

Figure 11 : Schematic illustration of microencapsulation method using chitosan and CMC as wall materials.

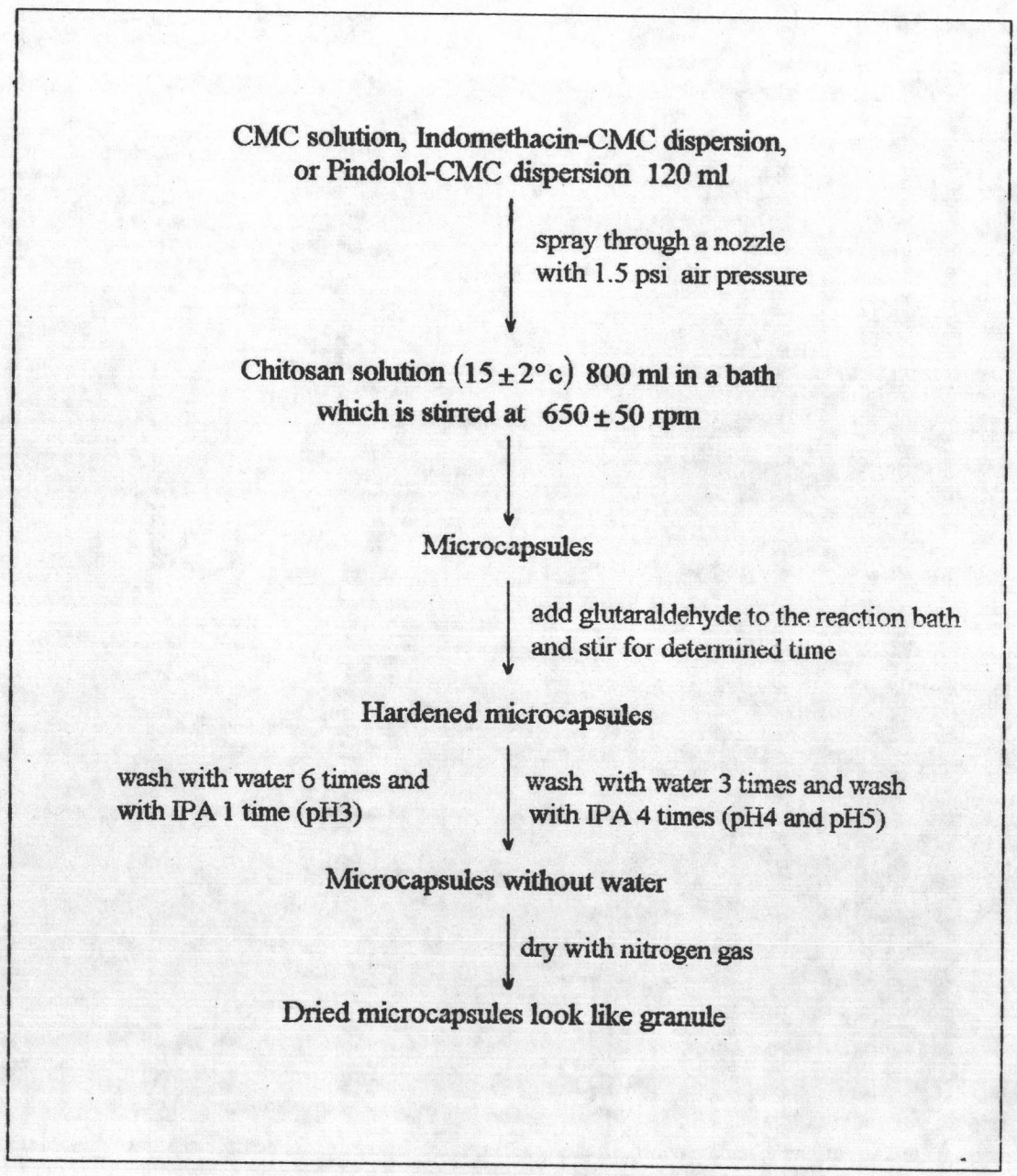

Table 4 : Variable factors in indomethacin microcapsules preparations

Preparation	[Chitosan soln] (% w/v)	pH of chitosan solution	Hardening Time (hr)	Glutaraldehyde /polymer 1gm (gm)
1	0.5	3	5	1.0
2	0.5	3	5	1.5
3	0.5	3	5	2.0
4	0.5	3	3	0.25
5	0.5	3	3	0.5
6	0.5	3	3	1.0
7	0.5	3	3	1.5
8	0.5	3	3	2.0
9	0.5	3	1	0.25
10	0.5	3	1	0.5
11	0.5	3	1	1.0
12	0.5	4	1	0.25
13	0.5	4	1	0.5
14	0.5	4	1	1.0
15	0.5	4	1	1.5
16	0.5	4	3	0.25
17	0.5	4	3	0.5
18	0.5	4	3	1.0
19	0.5	4	3	1.5
20	0.5	5	1	0.25
21	0.5	5	1	0.5
22	0.5	5	1	1.0
23	0.5	5	1	1.5
24	0.5	5	3	0.25
25	0.5	5	3	0.5
26	0.5	5	3	1.0
27	0.5	5	3	1.5
28	0.25	4	3	0.25
29	0.75	4	3	0.25
30	1.0	4	3	0.25

Table 5 : Variable pH of chitosan solution in pindolol microcapsules preparations

Preparation	[Chitosan soln] (% w/v)	pH of chitosan solution	Hardening Time (hr)	Glutaraldehyde /polymer 1gm (gm)
31	0.5	3	3	0.25
32	0.5	4	3	0.25
33	0.5	5	3	0.25

4. Evaluation of Pharmaceutical Microcapsule

4.1 Morphology

Morphology of microcapsules was determined by the use of scanning electron microscope (SEM), each of samples was coated with gold prior to the microscopic examination using ion sputtering. Shape and surface topography of microcapsules, before drug release, were observed at X75 and X750 magnifications. Selected preparations were observed for their surface topography of both before and after drug release at X7500 magnification. Drug entrapped in the microcapsules was determined by the use of optical microscope at X200 magnification. Selected samples were mounted with water prior to the microscopic examination.

4.2 Microcapsules Size and Size Distribution

Size and size distribution of microcapsules were determined by SEM. The particle size was determined by measuring the Feret's diameter, which was the distant between two tangents on opposite sides of the particle parallel to some fixed direction [Martin, 1993]. Sample size of 350 microcapsules was used for size distribution analysis. The number of particle size distribution was transformed into the percentage weight of particle size distribution. Then the cumulative percentage frequency undersize and normalised Z value (standard score) were calculated. Normalised Z value was transferred from cumulative percentage frequency undersize. In order to calculate the geometric mean diameter at $Z = 0$, (D_{50}) of microcapsule, particle diameter was transformed into logarithm value. The least square analysis was then employed to compute the D_{50} of microcapsule from the logarithm of particle diameter and Z value.

4.3 Determination of Indomethacin Content in Indomethacin Microcapsule

The method for determining indomethacin content employed in this study was modified from the USP XXII, monograph of Indomethacin-Extended-Release Capsule. Indomethacin microcapsules approximately 400 mg were grinded in mortar to destroy the microcapsules wall. Sample 120.0 mg was accurately weighed into a 100.0 ml volumetric flask. Then 60 ml of a solvent mixture, made up of methanol and pH 7.5 phosphate buffer (1:1) was added. The solution was then left to stand for 3 hours with occasional shaking. After standing, the volume was adjusted to 100.0 ml with the same solvent mixture, then the solution was mixed and filtered. The first 10 ml of filtrate was discarded and 1.0 ml was pippetted to a 25.0 ml volumetric flask, more solvent mixture was added to adjusted the volume. The absorbance of the resulting solution was measured at 322 nm, using a double beam spectrophotometer. The solvent mixture was employed as a blank. The indomethacin content was calculated from a calibration curve. Each sample was determined in triplicate.

4.4 Determination of Pindolol Content in Pindolol Microcapsule

The method for determining pindolol content employed in this study was modified from the USP XXII, monograph of Pindolol Tablet. The drug content of pindolol microcapsule was determined using the same method as for the indomethacin microcapsule, except the solvent mixture used was 7:3 methanol and chloroform, and the absorbance of the resulting solution was measured at 266 nm.

4.5 Drug Release Study of Indomethacin Microcapsule

Drug release study of indomethacin microcapsule was determined according to the USP XXII, using apparatus I (the basket method). Nine hundred millilitres of pH 6.2 phosphate buffer were placed into a glass vessel specified in the USP dissolution test, and equilibrated at 37±0.5°c. One capsule was placed in a dry basket, specified in the compendium, and immersed in the medium at the centre of the vessel and at 20 mm above the bottom of the vessel. The apparatus was operated at a speed of 75 rpm. Three capsules of each formulation were evaluated.

Five millilitres of specimen were withdrawn at the time interval of 15, 30 and 45 minutes, then 1, 2, 3, 5, 7, 9, 12, 15, 18 and 24 hours. The same quantity of medium was added immediately after each sampling to keep the volume of the medium constant during the experiment. The absorbance of each sample was determined by using a spectrophotometer at 320 nm. The sample was diluted to a suitable concentration if necessary.

The amount of indomethacin release at any time interval was calculated from the calibration curve. A cumulative correction was made for the previously removed sample to determine the total amount of drug release.

4.6 Calibration Curve of Indomethacin

4.6.1 In the mixture of methanol and pH 7.5 phosphate buffer (1:1)

Indomethacin 50 mg was accurately weighed and dissolved in the solvent mixture, a solvent mixture of methanol and pH 7.5 phosphate buffer (1:1). The solution was then adjusted to 100 ml and used as stock solution.

The stock solutions of 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 ml were individually pipetted into 100.0 ml volumetric flask and diluted to volume with the solvent mixture. The final concentration of each solution were 15.0, 20.0, 25.0, 30.0, 35.0 and 40.0 μg/ml respectively.

The absorbance of known drug concentration was determined by a double beam spectrophotometer in a 1-cm cell at 322 nm. The solvent mixture was used as a blank solution. Each concentration was determined in triplicate. The calibration curve of indomethacin was illustrated in Figure 67 in the Appendix.

4.6.2 In the pH 6.2 phosphate buffer

Indomethacin 50 mg was accurately weighed and dissolved in 8.0 ml of methanol The solution was then adjusted to 100.0 ml with pH 6.2 phosphate buffer and used as stock solution.

The stock solutions of 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 ml were individually pipetted into 100.0 ml volumetric flask and diluted to volume with pH 6.2 phosphate buffer. The final concentrations of each solution were 15.0, 20.0, 25.0, 30.0, 35.0 and 40.0 μg/ml respectively.

The absorbance of known drug concentration was determined by a double beam spectrophotometer in a 1-cm cell at 320 nm. pH 6.2 phosphate buffer was used as a blank solution. Each concentration was determined in triplicate. The calibration curve of indomethacin was illustrated in Figure 68 in the Appendix.

4.7 Calibration Curve of Pindolol

Pindolo 50 mg was accurately weighed and dissolved in the solvent mixture, a solvent mixture of methanol and chloroform (7:3). The solution was then adjusted to 200.0 ml and used as stock solution.

The stock solutions of 4.0, 5.0, 6.0, 7.0 and 8.0 ml were individually pipetted into 100.0 ml volumetric flask and diluted to volume with the solvent mixture. The final concentrations of each solution were 10.0, 12.5, 15.0, 17.5 and 20.0 $\mu g/ml$ respectively.

The absorbance of known drug concentration was determined by a double beam spectrophotometer in a 1-cm cell at 266 nm. The solvent mixture was used as a blank solution. Each concentration was determined in triplicate. The calibration curve of pindolol was illustrated in figure 69 in the Appendix.

4.8 Percentage of Drug Entrapment and Drug Recovery

From the determination of drug content in pharmaceutical microcapsule, percentage of drug entrapment and drug recovery were computed from the following formulas.

$$\% \text{ Drug Entrapment} = \frac{D_M \times 100}{120.0} \qquad (11)$$

$$\% \text{ Drug Recovery} = \frac{M_T \times D_M \times 100}{D_I \times 120.0} \qquad (12)$$

D_M = Drug content in the pharmaceutical microcapsule 120.0 mg (mg).

M_T = Total pharmaceutical microcapsule yield (gm) of each preparation.

D_I = The initial amount of drug used in the microencapsulation process (gm).

120.0 = The weight of microcapsule which was used in the determination of drug content in pharmaceutical microcapsule (mg).

4.9 Study on Reproducibility

Reproducibility of size, size distribution and drug release pattern of indomethacin microcapsules were investigated for three consecutive batches. Two preparations (conformed to the USP drug release specification shown in Table 6) were selected for this reproducibility study and were prepared according to conditions described in 3.2. Drug entrapment and drug recovery of indomethacin microcapsules of each batch were also tested and evaluated.

Table 6 : USP specification for indomethacin release at time interval

Time (hr)	Amount Dissolved
0.083 D	between 10% - 32%
0.167 D	between 20% - 52%
0.333 D	between 35% - 80%
1.000 D	not less than 60%
2.000 D	not less than 80%