

## Chapter III

### Results

#### 1. Preliminary study

Chitosan polymer could be dissolved in 1 %w/v of acetic acid to be yellowish transparent solution. Both CMC and pectin could be dispersed in water to be colloid of different viscosities depending on concentration. The viscosities of these polymer solutions and colloids are shown in Table 7. CMC colloid was transparent while opaque brown colloid was of pectin.

Chitosan-CMC and chitosan-pectin microcapsules could be prepared by complex coacervation technique. The processing conditions were fixed at pH 4.0, temperature of chitosan solution at  $10 \pm 2^\circ \text{C}$ , and various concentrations of chitosan, CMC or pectin solution. Preparations of indomethacin microcapsules by chitosan-CMC with different hardening times of 2 and 3 hours exhibited no difference in the percentage of drug entrapment and drug recovery as shown in Table 13. Therefore, the step of hardening time would be 2 hours in all preparations. Table 8 shows that no encapsulation was found in the preparation of 0.5%w/v CMC solution. However, other chitosan-CMC preparations could obviously form microcapsules as shown in Figure 5. For chitosan-pectin microencapsulation, it appeared as a slurry or non encapsulation when pectin solution was sprayed into chitosan solution. However, encapsulation occurred by adding calcium chloride into chitosan solution to precipitate and to prevent aggregation of the droplets of microcapsules. In Table 9, preparation of calcium chloride and pectin was non encapsulation. It showed

**Table 7 : Viscosities of chitosan, CMC, and pectin solutions in different concentrations at 25°c**

Polymer solution	Concentration (%w/v)	Viscosity at 25°c (cps)
Chitosan	0.25	<10
	0.50	<10
	0.75	<10
	1.00	<10
CMC	0.50	39.32
	0.75	82.94
	1.00	196.17
	1.50	906.85
Pectin	2.50	15.48
	5.00	79.63
	7.50	357.58
	10.00	1125.58

**Table 8 : Formability of chitosan-CMC microcapsules at various concentrations**

CMC solution (%w/v)	Chitosan solution (%w/v)			
	0.25	0.50	0.75	1.00
0.50	NE	NE	NE	NE
0.75	E	E	E	E
1.00	E	E	E	E
1.50	E	E	E	E

NE = Non Encapsulation, PE = Partial Encapsulation, E = Encapsulation



**Figure 5 : Optical photomicrographs and scanning electron photomicrographs of chitosan-CMC microcapsules prepared from chitosan 0.5%w/v, CMC 1%w/v, glutaral 0.25 gm, 2 hrs. hardening time**  
 (A and B = optical photomicrograph x100 and x200 magnifications  
 C and D = scanning electron photomicrograph x80 and x800 magnifications)

**Table 9 : Formability of chitosan-pectin microcapsules at various concentrations**

Pectin solution (%w/v)	Chitosan solution (%w/v) with calcium chloride 3 gm				
	0	0.25	0.50	0.75	1.00
2.50	NE	PE	PE	PE	NE
5.00	NE	E	E	E	E
7.50	NE	E	E	E	E
10.00	NE	E	E	E	E

NE = Non Encapsulation, PE = Partial Encapsulation, E = Encapsulation

only droplets of pectin solution in the calcium chloride solution. Pectin solution at the concentration of 2.5 %w/v in various concentration of chitosan-calcium chloride solution showed partial encapsulation. Table 10 describes the differences of microcapsules which were prepared by chitosan-CMC and chitosan-pectin during the process of spraying, washing and drying. In the process of spraying polymer into chitosan solution, the wall of chitosan-CMC microcapsule immediately and easily wrinkled while microcapsule wall of chitosan-pectin was unchanged. In addition, chitosan-CMC microcapsules could be washed and dried easier than chitosan-pectin microcapsules.

## 2. Microencapsulation of indomethacin

The dried chitosan-CMC indomethacin microcapsules looked like small particles of pale yellow to brown colour while the dried chitosan-pectin indomethacin microcapsules looked like granules of light brown colour. The dried

**Table 10 : Comparison of chitosan-CMC and chitosan-pectin microcapsules in each process**

Process	Chitosan-CMC	Chitosan-pectin
Spraying (Microencapsulation)	<ul style="list-style-type: none"> <li>- transparent microcapsules in colorless transparent solution</li> <li>- fast collapsing microcapsule wall</li> </ul>	<ul style="list-style-type: none"> <li>- transparent brownish microcapsules in opaque brown liquid</li> <li>- long period for collapsing microcapsule wall</li> </ul>
Washing	<ul style="list-style-type: none"> <li>- easy to wash</li> <li>- needed small volume of IPA to remove water in the microcapsules</li> </ul>	<ul style="list-style-type: none"> <li>- difficult to wash</li> <li>- needed large volume of IPA to remove water in the microcapsules</li> </ul>
Drying	<ul style="list-style-type: none"> <li>- fast drying (spray nitrogen gas about 30-60 minutes)</li> </ul>	<ul style="list-style-type: none"> <li>- long time for drying (spray nitrogen gas about 60-90 min and keep in dessicator at room temp. for 24-48 hours)</li> </ul>

chitosan-CMC indomethacin microcapsules seemed to be bulkier than chitosan-pectin microcapsules.

All preparations shown in Tables 5 and 6 could form microcapsules. However, some preparations failed to give collected yield in the recovery process because in the process of hardening microcapsule wall, the medium turned to gel. Preparations which failed in recovery process are shown in Table 11.

Table 11 : Appearance of indomethacin microcapsules which failed in recovery process

Prep.	Chitosan soln (%w/v)	Pectin soln (%w/v)	CaCl <sub>2</sub> (gm)/chitosan 1 gm	Glutaral(gm)/polymer 1 gm	Microcapsule appearance
29	0.50	5.0	3.0	0.25	Deposited in gel like medium
31	0.50	7.5	1.0	0.25	
37	0.50	7.5	3.0	0.25	
42d	0.75	10.0	3.0	0.10	
43c,d	0.75	10.0	3.0	0.25	

### 2.1 Morphology of indomethacin microcapsules

The optical photomicrographs of chitosan-pectin indomethacin microcapsule while pectin-indomethacin dispersion was being sprayed into chitosan-calcium chloride solution are shown in Figures 6-9. Figures 6 and 7 show preparations of chitosan 0.25%, various pectin concentrations at 5.0% and 7.5%, and various calcium chloride contents at 1 gm and 3 gm /gm chitosan and Figures 8 and 9 show preparations of chitosan 0.5% varied in the same condition. The photomicrographs showed that chitosan-pectin complex coacervation entrapped indomethacin in all preparations. However, the preparation which had only 1 gm of calcium chloride showed agglomeration of microcapsule droplets.

Figures 10-11 and 12-17 show the optical photomicrographs of dried chitosan-CMC and chitosan-pectin indomethacin microcapsules, respectively, prepared from various conditions and mounted with water prior to the microscopic examination at x200 magnification. The photomicrographs of Figures 10 and 11 showed that indomethacin was encapsulated in the transparent wrinkle microcapsule

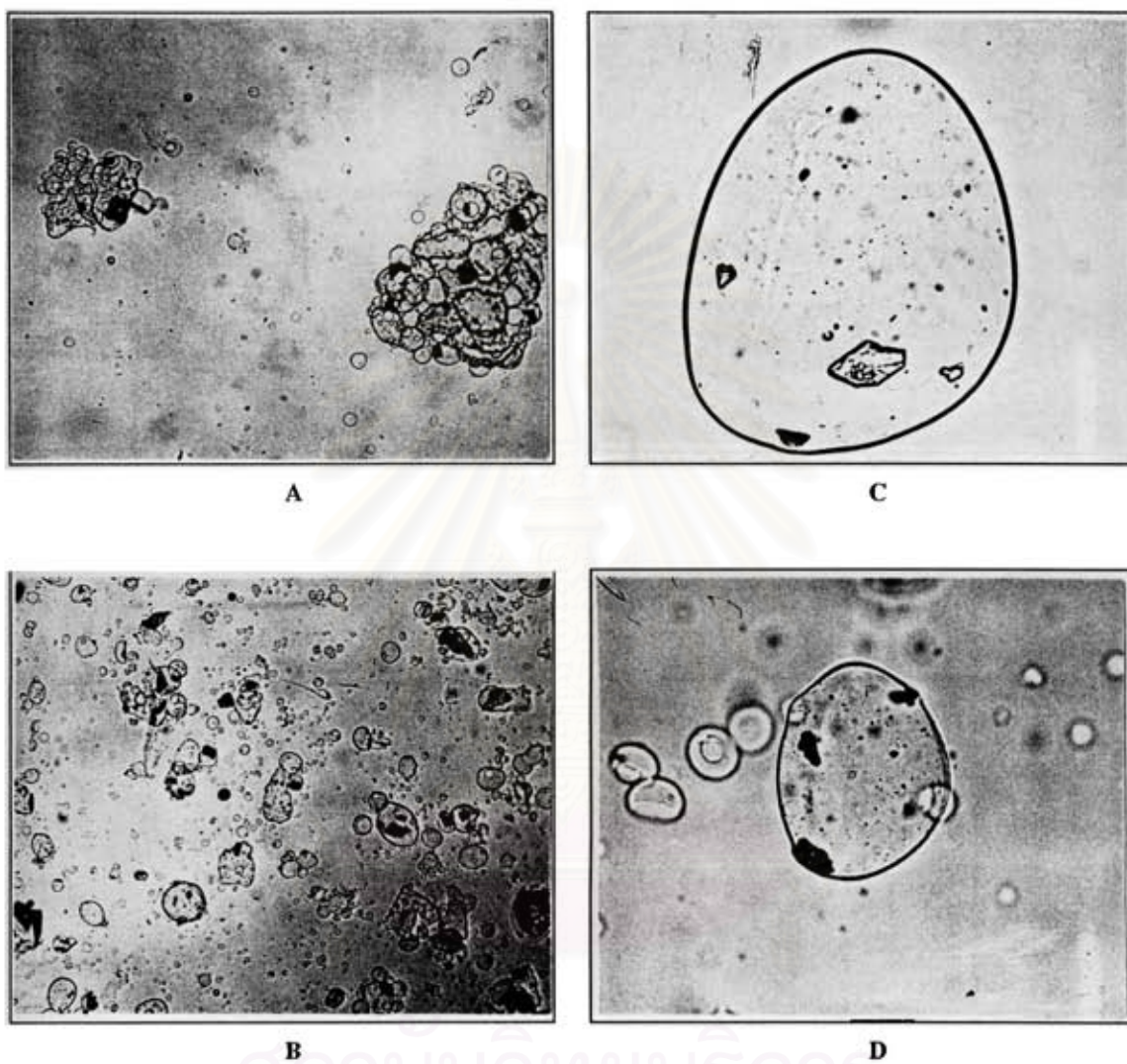
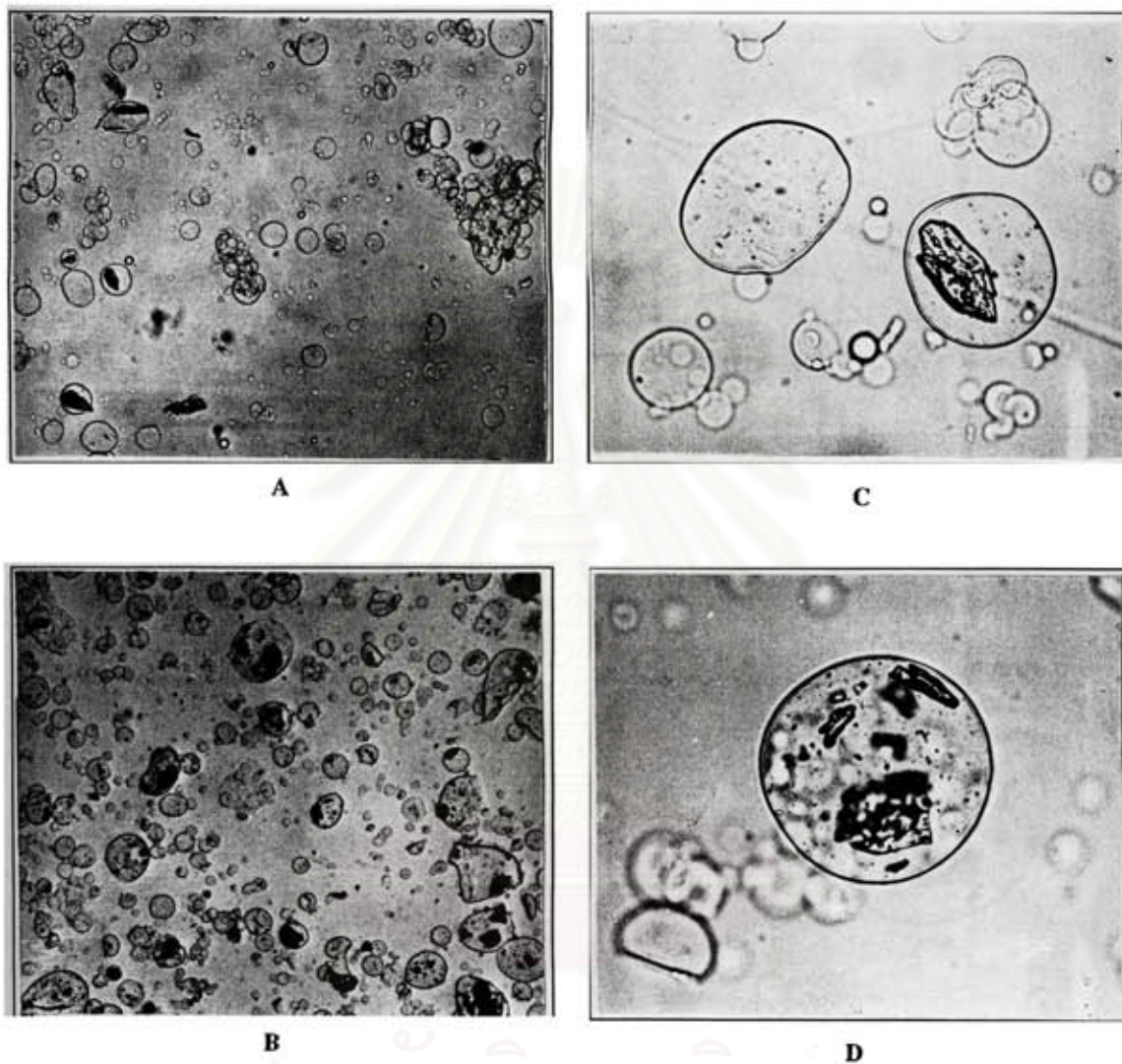


Figure 6 : Optical photomicrographs of chitosan-pectin indomethacin microencapsulation prepared

from chitosan 0.25%w/v, pectin 5%w/v

(A and C = calcium chloride 1 gm, B and D = calcium chloride 3 gm

A,B and C,D = x40 and x200 magnifications)



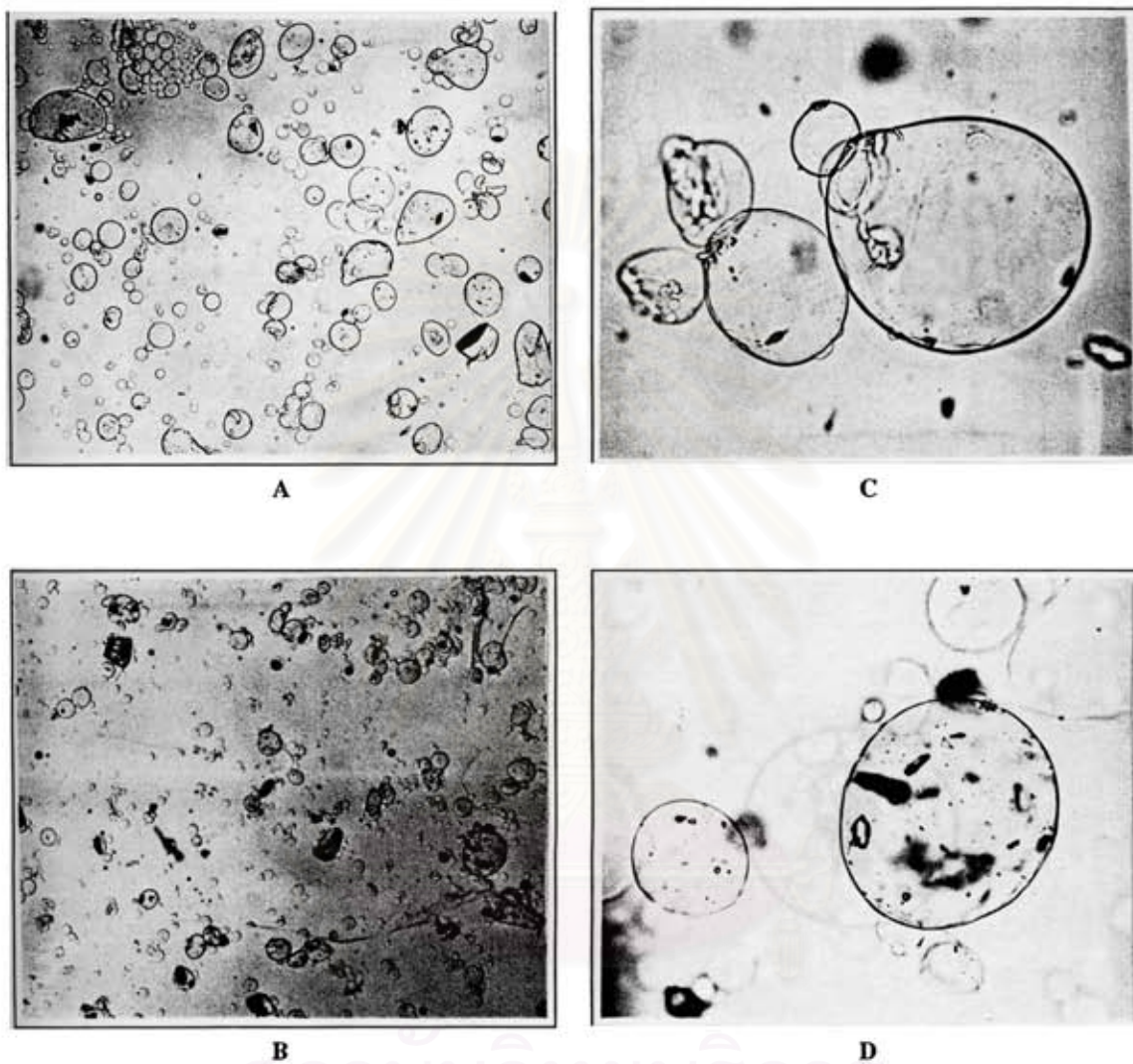
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**Figure 7 : Optical photomicrographs of chitosan-pectin indomethacin microencapsulation prepared from chitosan 0.25%w/v, pectin 7.5%w/v**

**(A and C = calcium chloride 1 gm, B and D = calcium chloride 3 gm**

**A,B and C,D = x40 and x200 magnifications)**





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**Figure 8 : Optical photomicrographs of chitosan-pectin indomethacin microencapsulation prepared from chitosan 0.5%w/v, pectin 5%w/v (A and C = calcium chloride 1 gm, B and D = calcium chloride 3 gm A,B and C,D = x40 and x200 magnifications)**

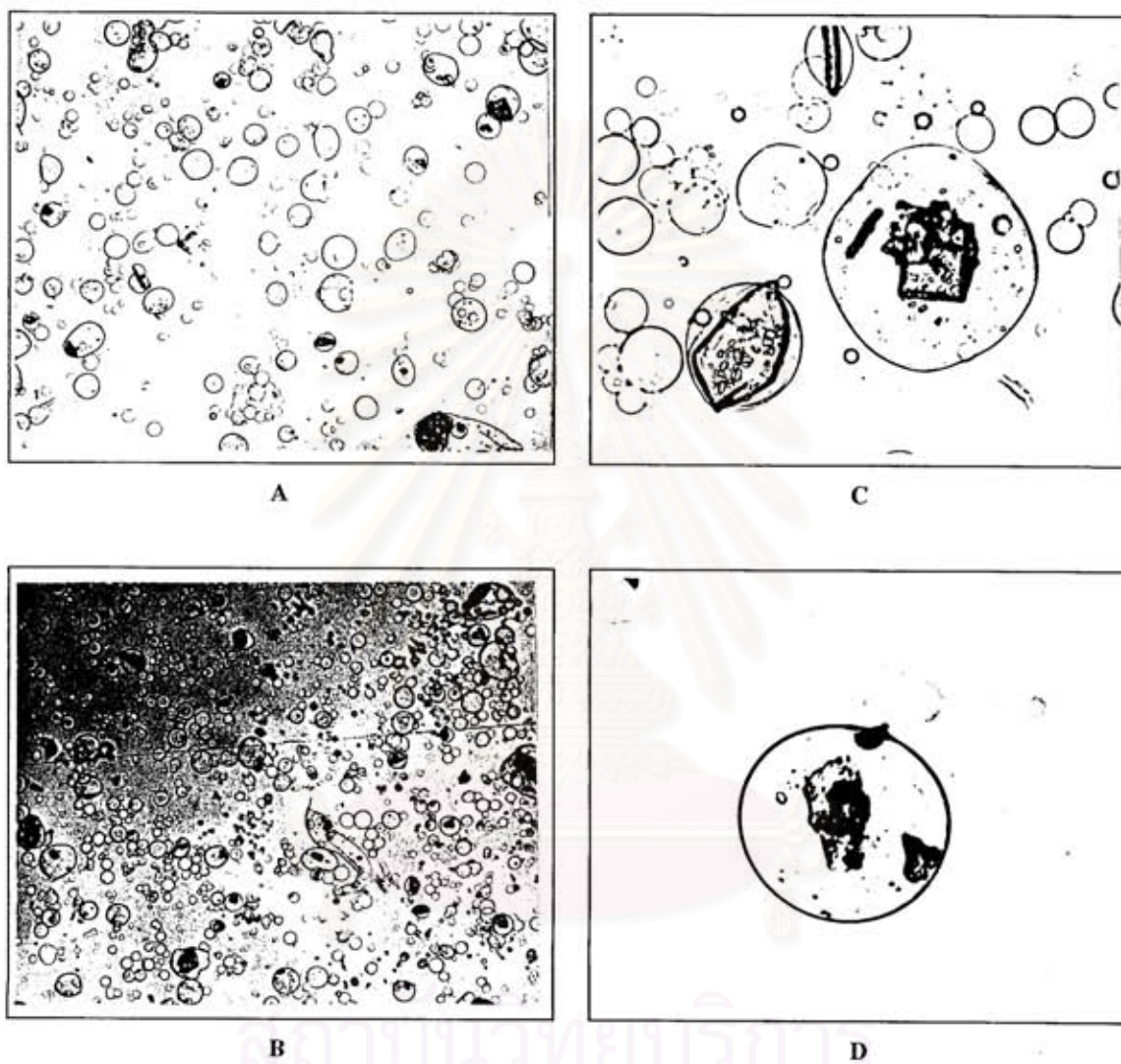


Figure 9 : Optical photomicrographs of chitosan-pectin indomethacin microencapsulation prepared from chitosan 0.5%w/v, pectin 7.5%w/v (A and C = calcium chloride 1 gm, B and D = calcium chloride 3 gm (A,B and C,D = x40 and x200 magnifications)

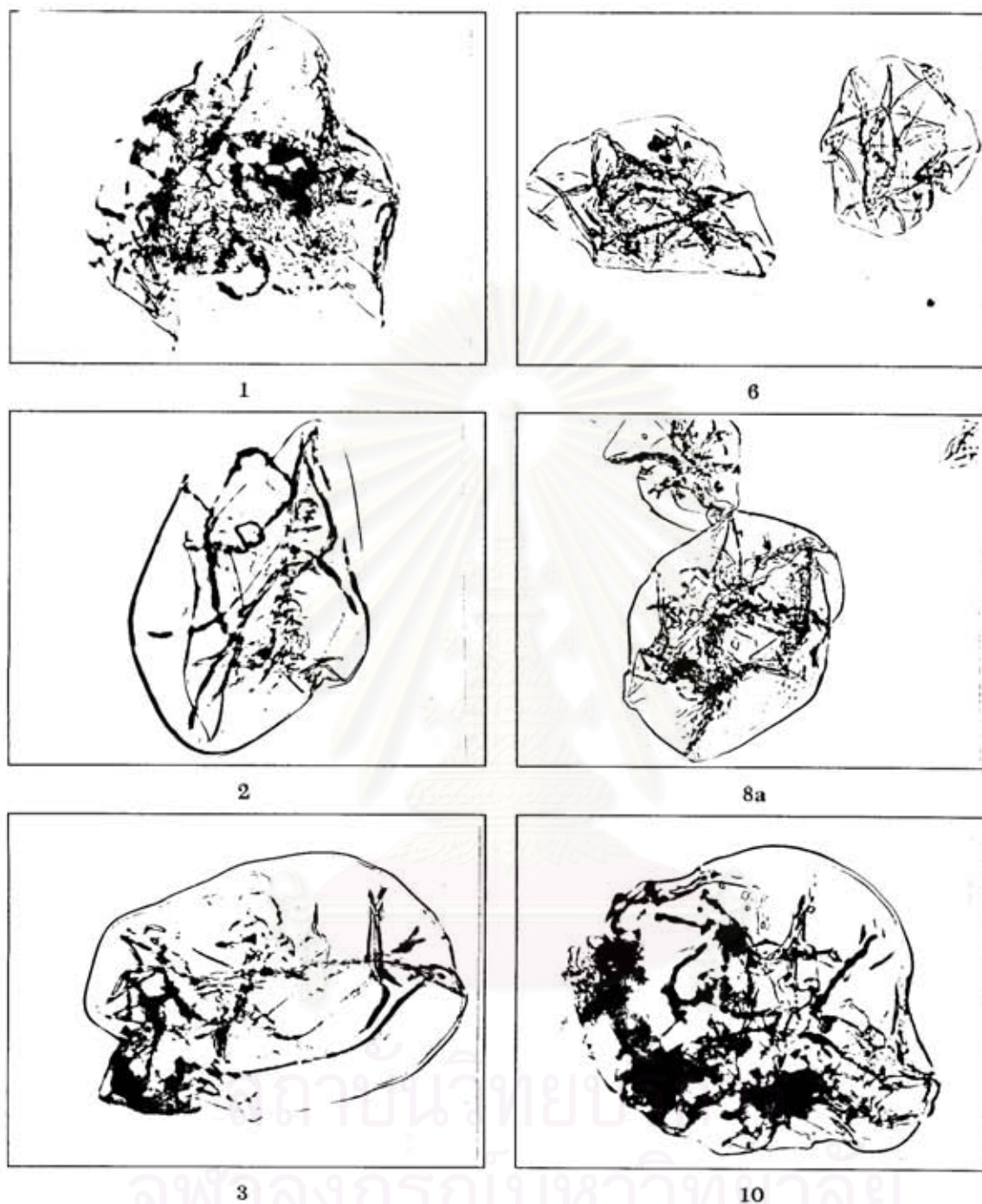
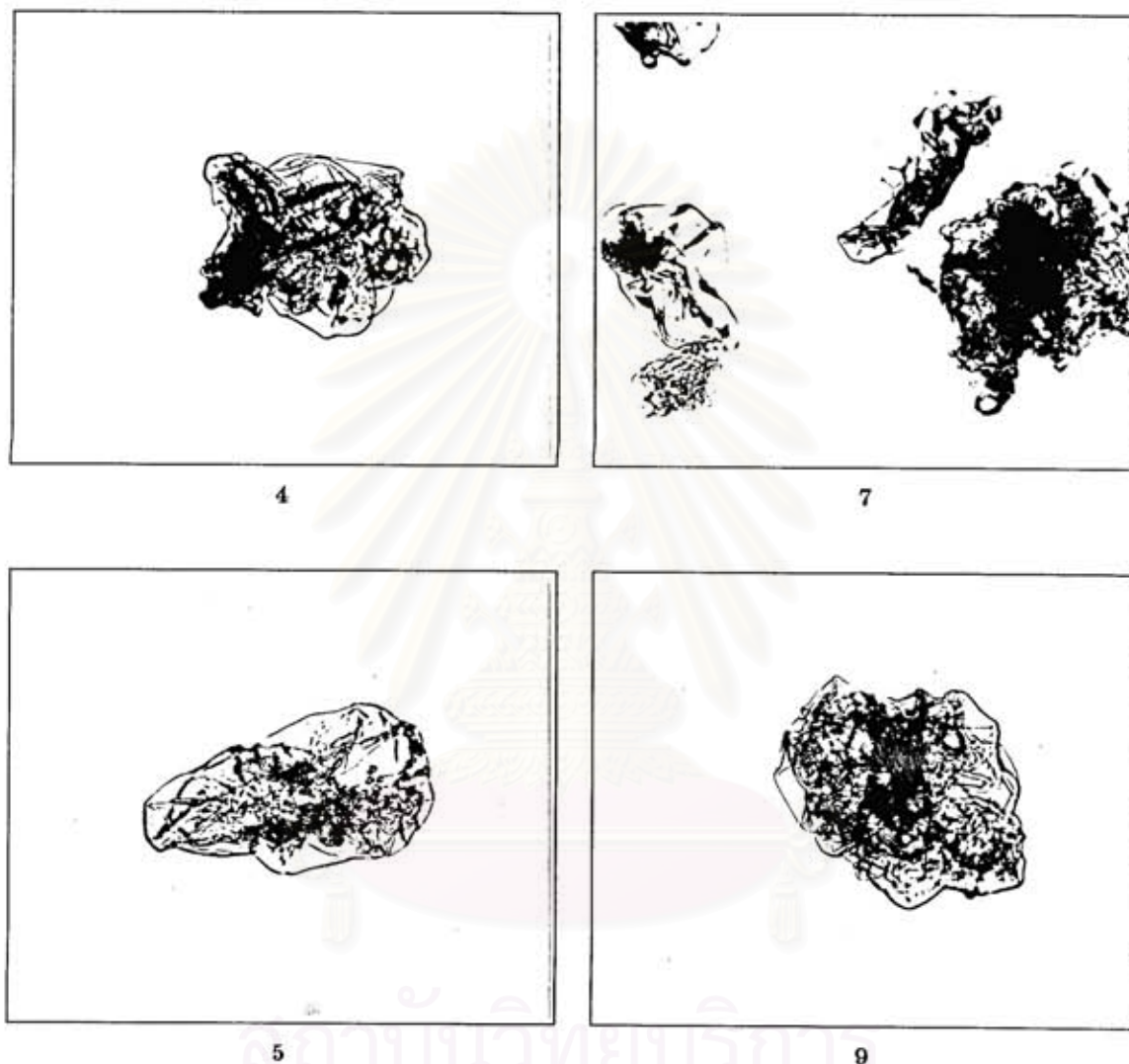


Figure 10 : Optical photomicrographs of chitosan-CMC indomethacin microcapsules prepared from glutaral 0.25 gm, 2 hrs. hardening time (prep. 1,2,3,6,8a,10), mounted with water, x200 magnification

(1 = CS 0.25 %w/v, CMC 0.75%w/v    6 = CS 0.5%w/v, CMC 0.75%w/v

2 = CS 0.25 %w/v, CMC 1%w/v        8a = CS 0.5 %w/v, CMC 1%w/v

3 = CS 0.25 %w/v, CMC 1.5 %w/v    10 = CS 0.5 %w/v, CMC 1.5 %w/v)



**Figure 11 : Optical photomicrographs of chitosan-CMC indomethacin microcapsules prepared from 2 hrs. hardening time (prep.4,5,7,9), mounted with water, x200 magnification**

**(4 = CS 0.35%w/v, CMC 1%w/v, glutaral 0.25 gm, indomethacin 1.5%**

**5 = CS 0.5%w/v, CMC 0.75%w/v, glutaral 0.1 gm, indomethacin 1%**

**7 = CS 0.5%w/v, CMC 1%w/v, glutaral 0.1 gm, indomethacin 1%**

**9 = CS 0.5%w/v, CMC 1%w/v, glutaral 0.25 gm, indomethacin 1.5%)**

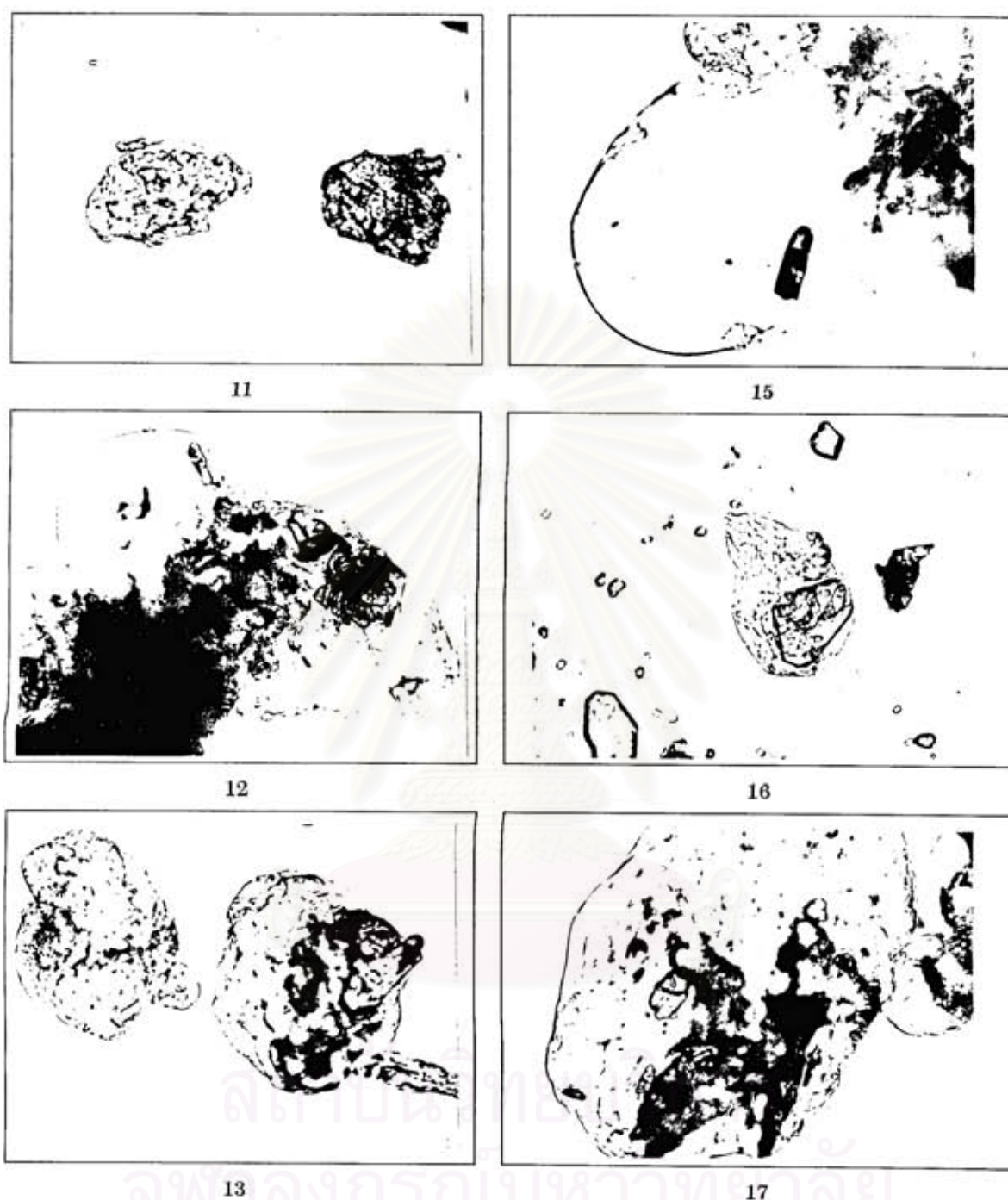


Figure 12 : Optical photomicrographs of chitosan-pectin indomethacin microcapsules prepared from 2 hrs. hardening time (prep.11,12,13,15,16,17), mounted with water, x200 magnification

11=CS 0.25%,pectin 5 %w/v,CaCl<sub>2</sub> 1 gm, glutaral 0.1 gm,indomethacin 1%

12=CS 0.25%,pectin 5 %w/v,CaCl<sub>2</sub> 1 gm, glutaral 0.1 gm,indomethacin 2%

13=CS 0.25%,pectin 5 %w/v,CaCl<sub>2</sub> 1 gm, glutaral 0.25 gm,indomethacin 1%

15=CS 0.25%,pectin 5 %w/v,CaCl<sub>2</sub> 3 gm, glutaral 0.1 gm,indomethacin 1%

16=CS 0.25%,pectin 5 %w/v,CaCl<sub>2</sub> 3 gm, glutaral 0.25 gm,indomethacin 1%

17=CS 0.25%,pectin 5 %w/v,CaCl<sub>2</sub> 3 gm, glutaral 0.25 gm,indomethacin 2%)

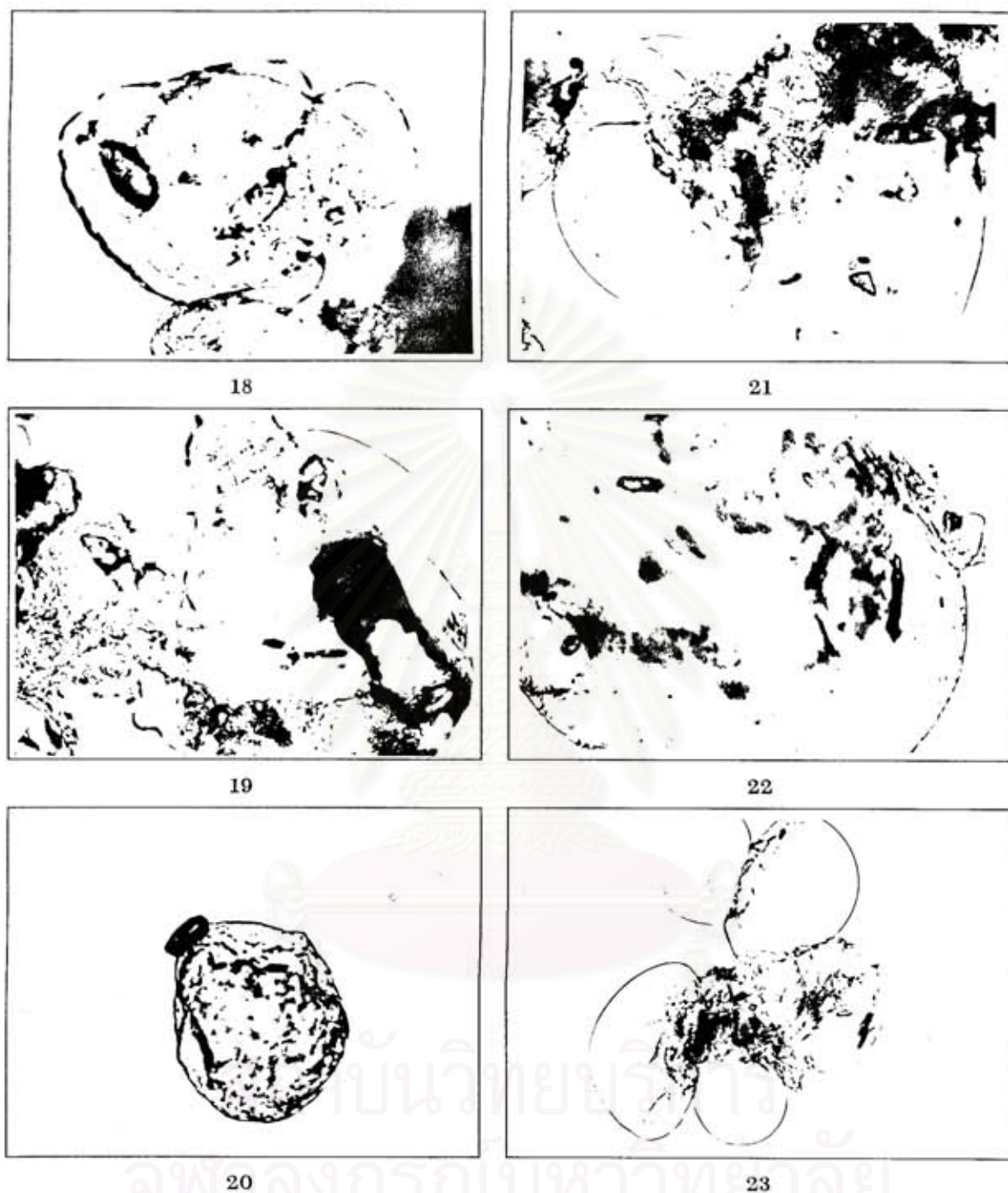


Figure 13 : Optical photomicrographs of chitosan-pectin indomethacin microcapsules prepared from 2 hrs. hardening time (prep.18,19,20,21,22,23), mounted with water, x200 magnification

18=CS 0.25 %,pectin 7.5 %w/v,CaCl<sub>2</sub> 1 gm, glutaral 0.1 gm,indomethacin 1%

19=CS 0.25 %,pectin 7.5 %w/v,CaCl<sub>2</sub> 1 gm, glutaral 0.1 gm,indomethacin 2%

20=CS 0.25 %,pectin 7.5 %w/v,CaCl<sub>2</sub> 1 gm, glutaral 0.25 gm,indomethacin 1%

21=CS 0.25 %,pectin 7.5 %w/v,CaCl<sub>2</sub> 3 gm, glutaral 0.1 gm,indomethacin 1%

22=CS 0.25 %,pectin 7.5 %w/v,CaCl<sub>2</sub> 3 gm, glutaral 0.25 gm,indomethacin 1%

23=CS 0.25 %,pectin 7.5 %w/v,CaCl<sub>2</sub> 3 gm, glutaral 0.25 gm,indomethacin 2%)

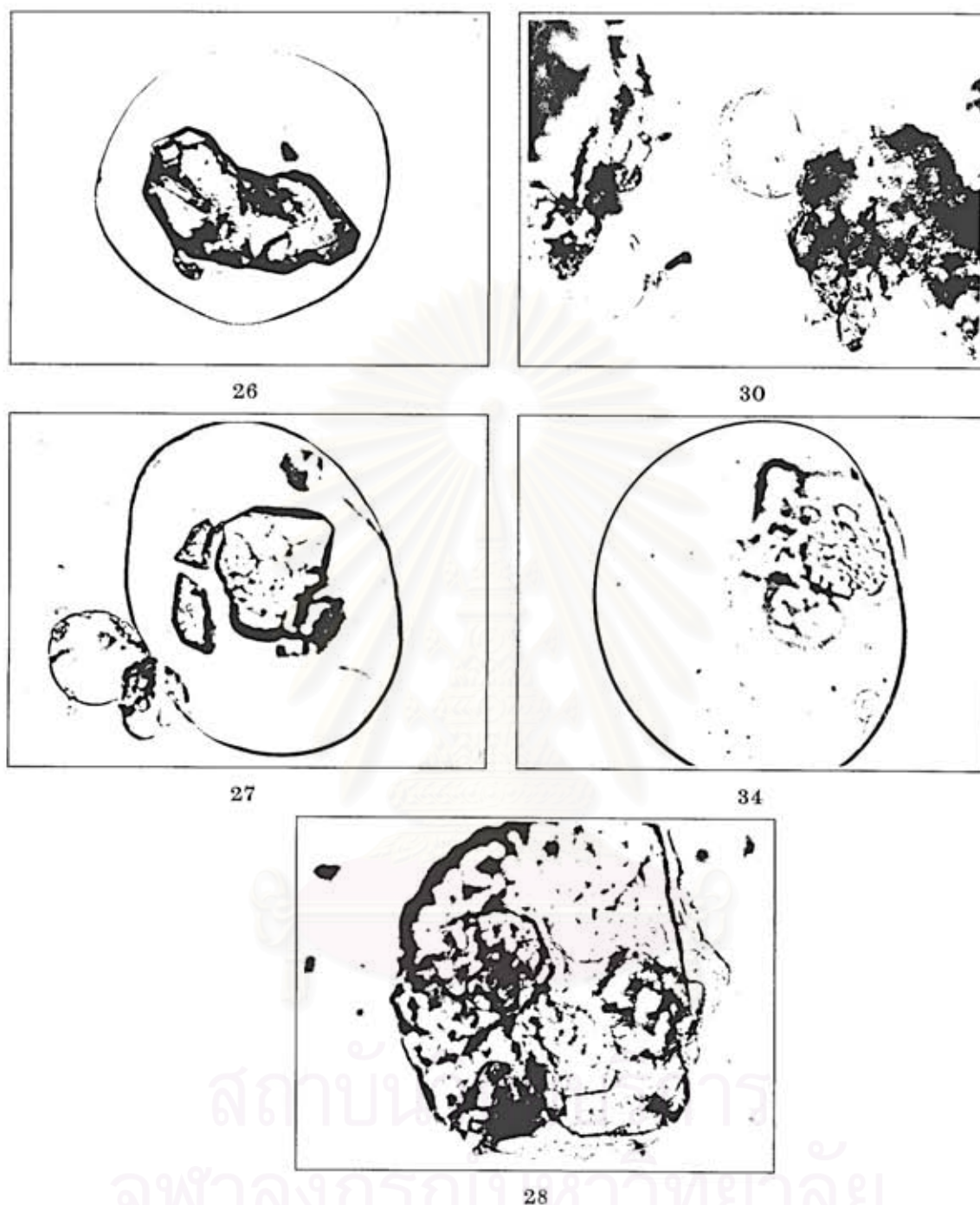


Figure 14 : Optical photomicrographs of chitosan-pectin indomethacin microcapsules prepared from 2 hrs. hardening time (prep.26,27,28,30,34), mounted with water, x200 magnification

(26=CS 0.5%,pectin 5%w/v,CaCl<sub>2</sub> 1 gm, glutaral 0.1 gm,indomethacin 1%

27=CS 0.5%,pectin 5%w/v,CaCl<sub>2</sub> 1 gm, glutaral 0.25 gm,indomethacin 1%

28=CS 0.5%,pectin 5%w/v,CaCl<sub>2</sub> 3 gm, glutaral 0.1 gm,indomethacin 1%

30=CS 0.5%,pectin 7.5%w/v,CaCl<sub>2</sub> 1 gm, glutaral 0.1 gm,indomethacin 1%

34=CS 0.5%,pectin 7.5%w/v,CaCl<sub>2</sub> 3 gm, glutaral 0.1 gm,indomethacin 1%)

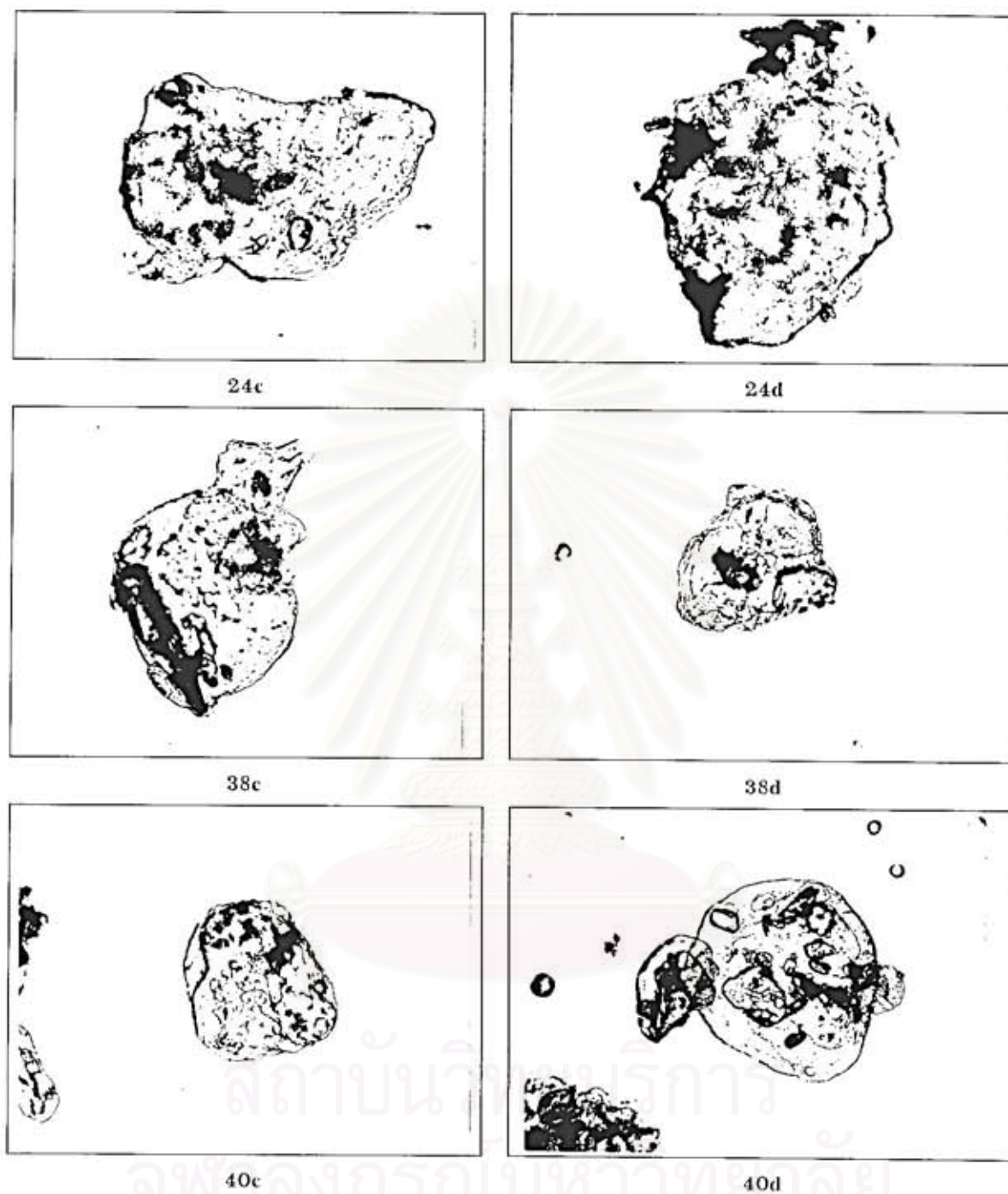


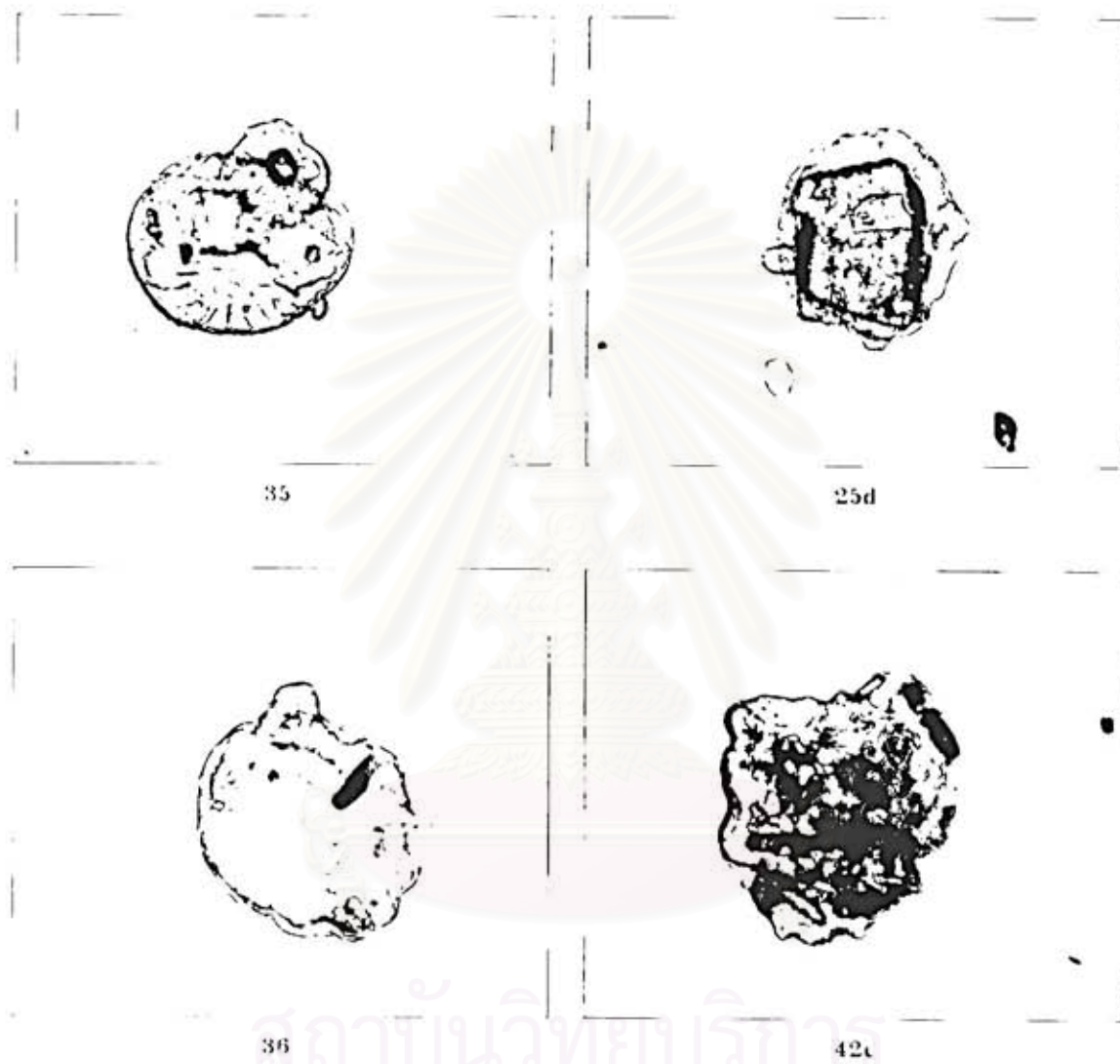
Figure 15 : Optical photomicrographs of chitosan-pectin indomethacin microcapsules prepared from calcium chloride 3 gm, glutaral 0.25 gm, indomethacin 3% (prep. 24c, 24d, 38c, 38d, 40c, 40d), mounted with water, x200 magnification

(24c and 24d = CS 0.25%, pectin 7.5%w/v, 6 and 18 hrs. hardening time

38c and 38d = CS 0.5%, pectin 7.5%w/v, 6 and 18 hrs. hardening time

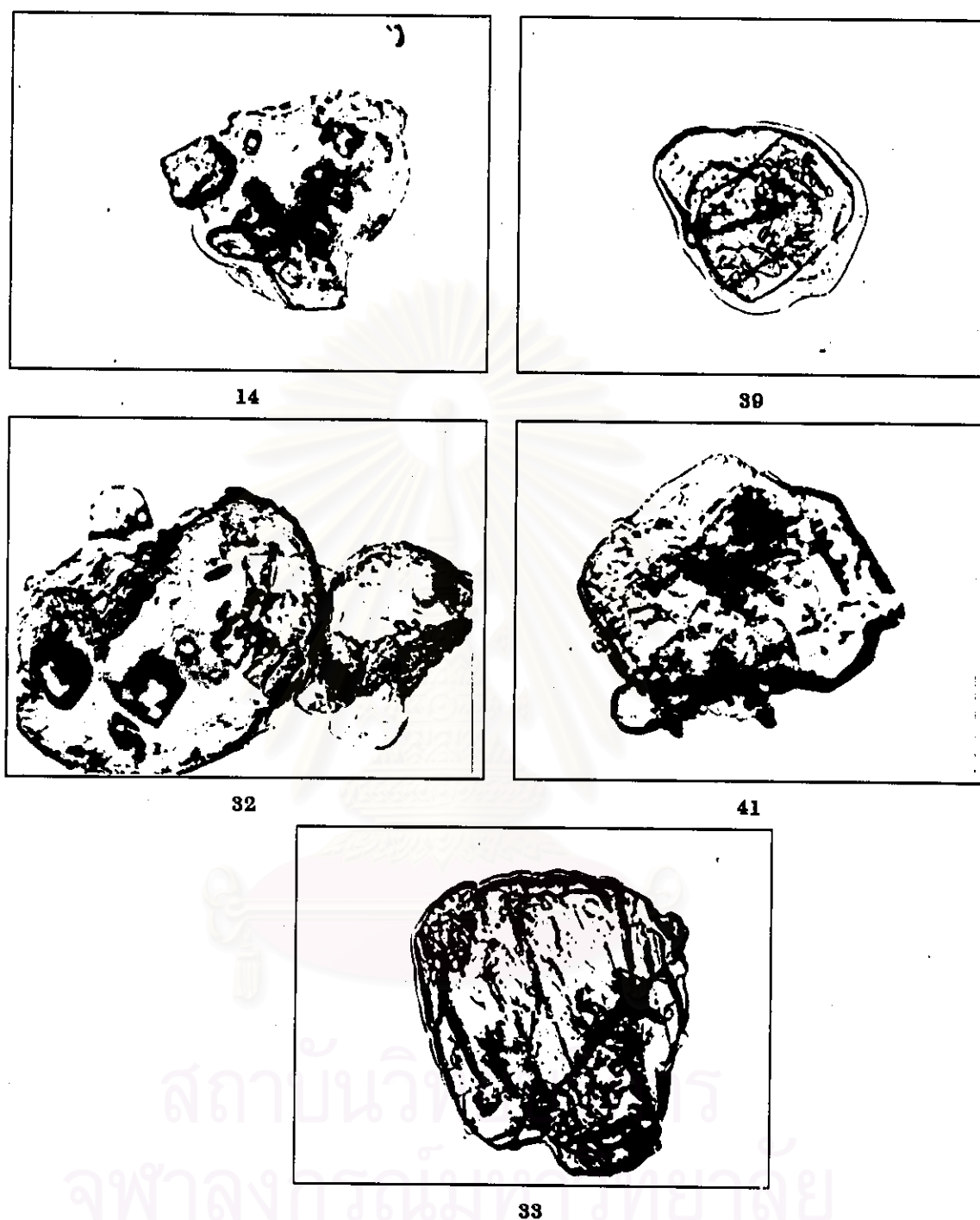
40c and 40d = CS 0.5%, pectin 10%w/v, 6 and 18 hrs. hardening time)





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Figure 16 + Optical photomicrographs of chitosan-pectin indomethacin microcapsules (prep.35,36, 25d,42c), mounted with water, x200 magnification  
 :35 and 36-CS 0.5%,pectin 7.5% w/v, CaCl<sub>2</sub> 3 gm,glutaral 0.1 gm,indomethacin 2 and 3%  
 25d = CS 0.25% , pectin10%,CaCl<sub>2</sub> 3 gm,glutaral 0.25 gm,indo.3%,18 hrs. hardening time)  
 42c = CS 0.75% , pectin 10%,CaCl<sub>2</sub> 3 gm,glutaral 0.1 gm,indo.3%,6 hrs. hardening time)



**Figure 17 : Optical photomicrographs of chitosan-pectin indomethacin microcapsules prepared from no glutaral (prep.14,32,33,39,41), mounted with water, x200 magnification**

**(14 = chitosan 0.25%, pectin 5% w/v, CaCl<sub>2</sub> 3 gm, indomethacin 3%**

**32 and 33 = chitosan 0.5%, pectin 7.5% w/v, CaCl<sub>2</sub> 3 gm, indomethacin 1 and 5%**

**39 = chitosan 0.5%, pectin 10% w/v, CaCl<sub>2</sub> 3 gm, indomethacin 3%**

**41 = chitosan 0.75%, pectin 10% w/v, CaCl<sub>2</sub> 3 gm, indomethacin 3%)**

wall of chitosan-CMC. When mounted with water, the chitosan-CMC microcapsule wall was slightly swollen. In Figure 10, higher concentrations of CMC showed thicker microcapsule wall. In Figure 11, preparations 5 and 7 which had less glutaraldehyde of 0.1 gm /gm polymer showed weak and partially complete of microcapsule wall when compared to preparations 6 and 8a, respectively. When more indomethacin was loaded in the preparation 9, more drug could be seen in microcapsule than in preparation 8a.

Figures 12-17 show the optical photomicrographs of chitosan-pectin indomethacin microcapsules mounted with water. The wall was transparent and indomethacin was entrapped inside. Figures 12-13 and 14 display optical photomicrograph of various preparations which prepared from chitosan 0.25% and 0.5 %w/v, respectively, various using pectin 5% and 7.5 %w/v,  $\text{CaCl}_2$  1 and 3 gm / gm chitosan, glutaraldehyde 0.1 and 0.25 gm /gm polymer, indomethacin 1% and 2%. Figure 15 shows optical photomicrograph of preparations with hardening time of 6 and 18 hours which prepared from chitosan 0.25% and 0.5 %w/v, pectin 7.5% and 10.0 %w/v,  $\text{CaCl}_2$  3 gm /gm chitosan, glutaraldehyde 0.25 gm /gm polymer, indomethacin 3%. The optical photomicrographs of preparations 35, 36, 25d, and 42c are shown in Figure 16. The optical photomicrographs of Figures 12-16 show notably more swollen of chitosan-pectin microcapsule wall. But optical photomicrographs in Figure 17 of preparations without glutaraldehyde show only slightly swollen microcapsule wall. Moreover, microcapsules of some preparations fused into large particles hence they were opaque when mounted with water.

The scanning electron photomicrographs of chitosan, CMC, pectin, and indomethacin are shown in Figure 18. Irregular shapes of particles were observed in chitosan, CMC, and pectin. Regular crystalline in various size was of

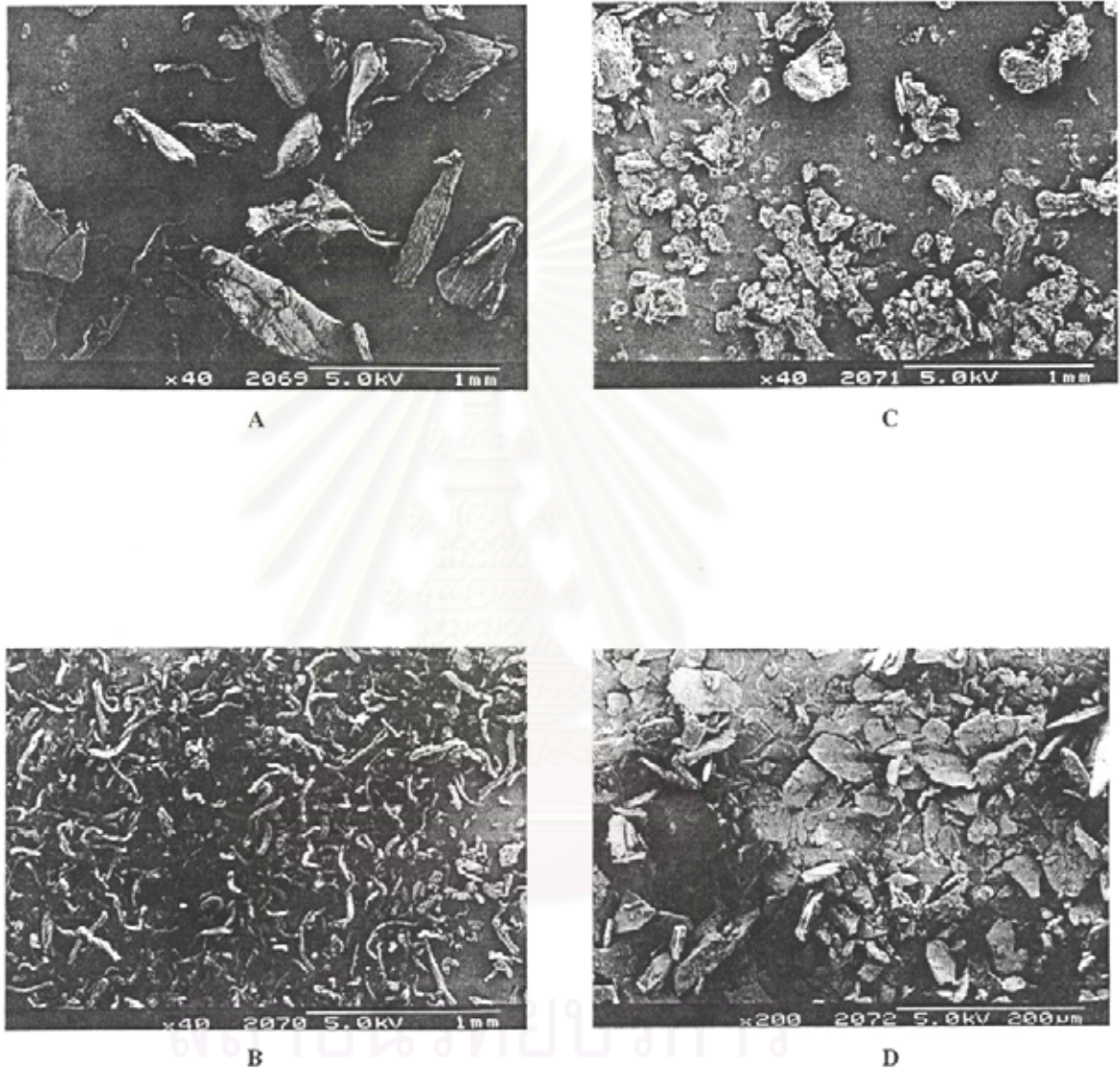


Figure 18 : Scanning electron photomicrographs of chitosan(A), CMC(B), pectin(C), x40 magnification, and indomethacin(D) x200 magnification

indomethacin.

Figures 19-22 and 23-35 show the shape and surface topography of chitosan-CMC and chitosan-pectin indomethacin microcapsules, respectively, prepared from different conditions. Figures 19 and 20 compare the shape and surface of chitosan-CMC indomethacin microcapsule prepared with chitosan of 0.25% and 0.5 %w/v and the CMC concentrations of 0.75%, 1.0%, and 1.5 %w/v. The shape was partially round. The membrane surface was smooth to wavy, folding and no pore. It was notable that shape and surface of microcapsules using various chitosan concentrations seemed to be no difference as shown in Figures 19&20, and 22. Using various CMC concentrations as shown in Figures 19-21 showed differences in thickness and creasing of membranes. Higher CMC concentration caused thicker and wavier membrane than lower concentration. It was shown in Figure 21 that surface and shape of microcapsule of the preparations with 0.1 gm of glutaraldehyde were thin and showed some crackings on the membrane when compared to the surface and shape of preparations 6 and 8a in Figure 20.

The shape and surface topography of chitosan-pectin indomethacin microcapsule are shown in Figures 23-35. Traces of indomethacin could be seen on the surface of microcapsule in all preparations. It could be observed in Figures 31-35 of preparations 24c-25d and 38c-42c that the shape was not round, moreover, it had a heavily wavy form and the surface of membrane was rough, more threads or fibers in a form of net with no pore. The topograph of preparations of higher chitosan and pectin concentration showed thicker membrane. However, the topographs of preparation with  $\text{CaCl}_2$  3 gm /gm chitosan and 2 hours hardening time shown in Figures 24, 26, 27 Prep.28, 28 Prep.34, 29-30 were unround shape, wavy surface of microcapsule membrane and some agglomeration of



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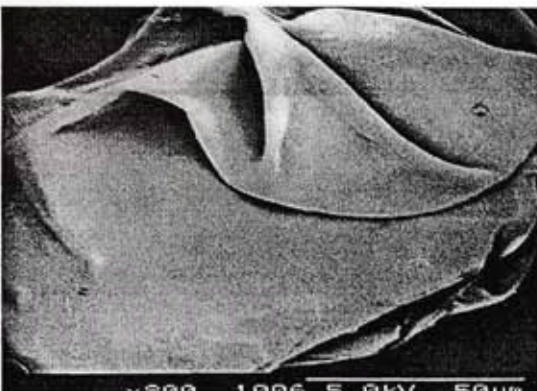
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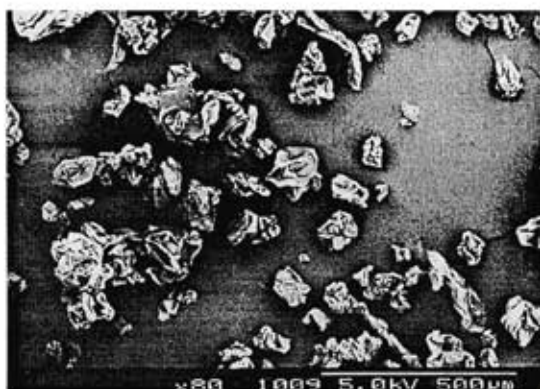


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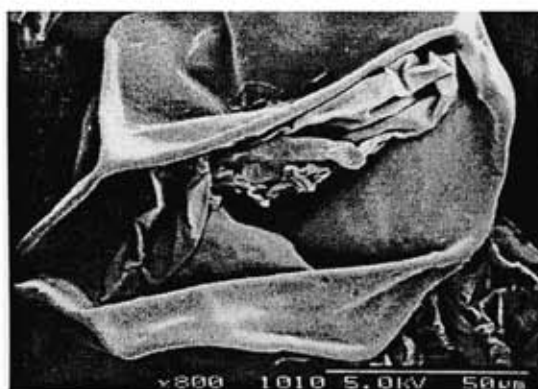


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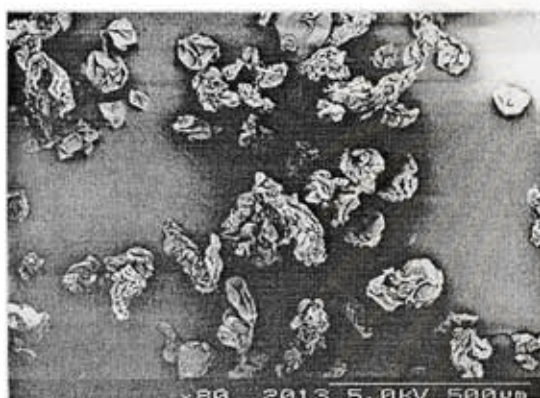
Figure 19 : Scanning electron photomicrographs of chitosan-CMC indomethacin microcapsules prepared from chitosan 0.25%w/v, glutaral 0.25 gm, 2hrs. hardening time (prep.1,2,3), x80 and x800 magnification  
(1 = CMC 0.75%w/v, 2 = CMC 1.0%w/v, 3 = CMC 1.5%w/v)



6



6



8a



8a

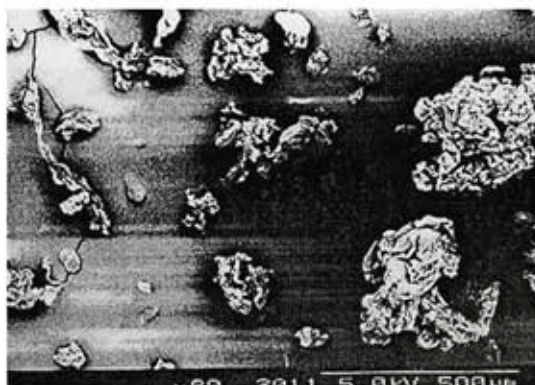


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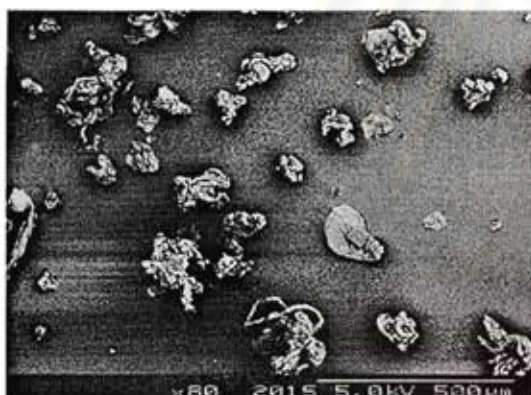
Figure 20 : Scanning electron photomicrographs of chitosan-CMC indomethacin microcapsules prepared from chitosan 0.5%w/v, glutaral 0.25 gm, 2hrs. hardening time (prep.6,8a,10), x80 and x800 magnification (6 = CMC 0.75 %w/v, 8a = CMC 1.0%w/v, 10 = CMC 1.5 %w/v)



5



5



7



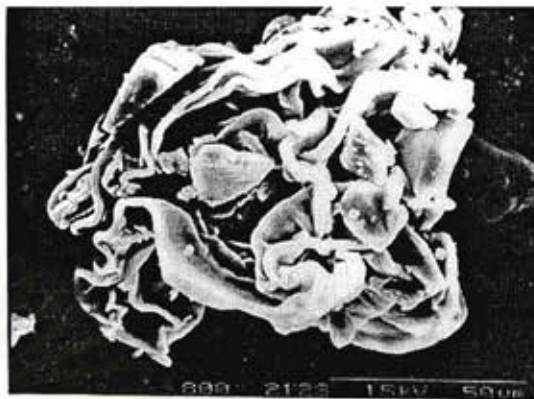
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**Figure 21 : Scanning electron photomicrographs of chitosan-CMC indomethacin microcapsules prepared from chitosan 0.5%w/v, glutaral 0.1 gm, 2hrs. hardening time (prep.5,7), x80 and x800 magnification (5 = CMC 0.75%w/v, 7 = CMC 1.0%w/v)**

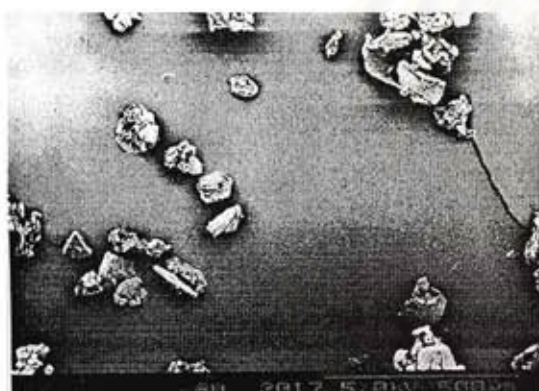




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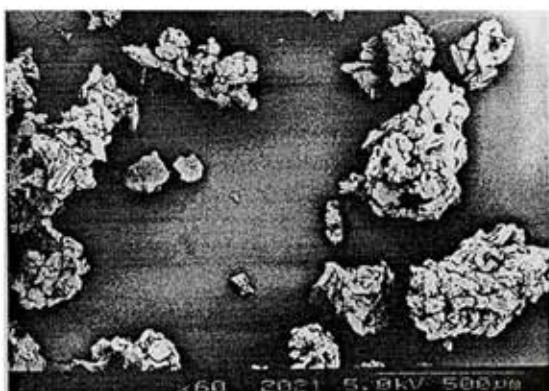


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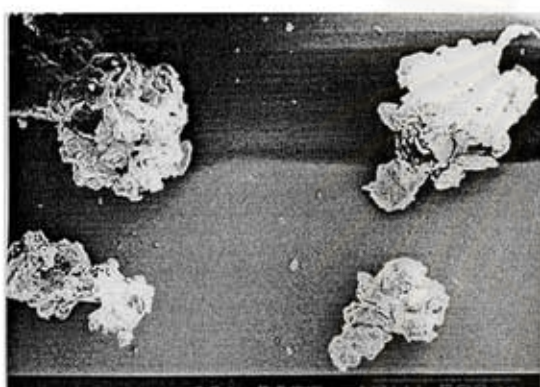
Figure 22 : Scanning electron photomicrographs of chitosan-CMC indomethacin microcapsules prepared from CMC 1.0%w/v, glutaral 0.25 gm, indomethacin 1.5%, 2 hrs. hardening time (prep.4,9), x80 and x800 magnification (4 = chitosan 0.35%w/v, 9 = chitosan 0.5%w/v)



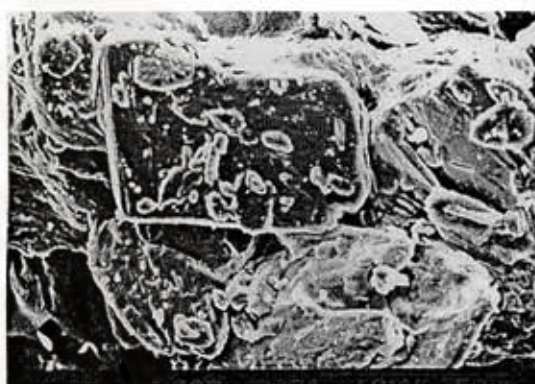
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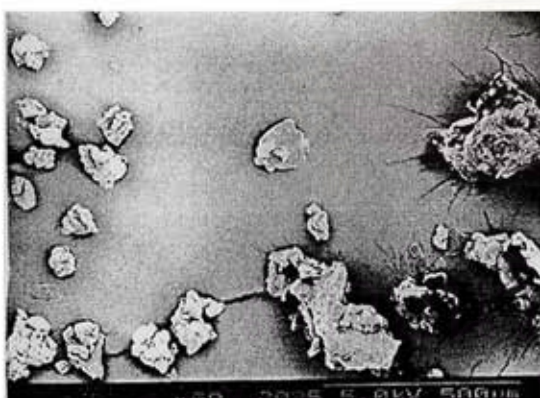
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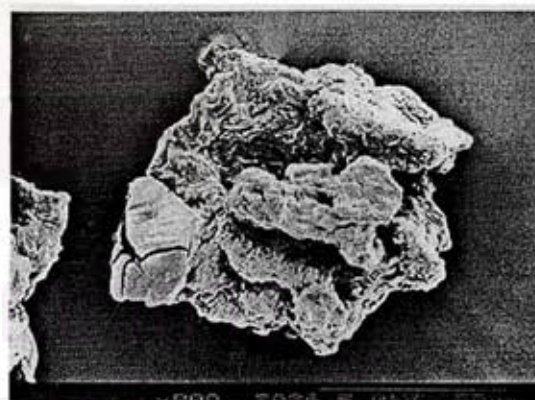
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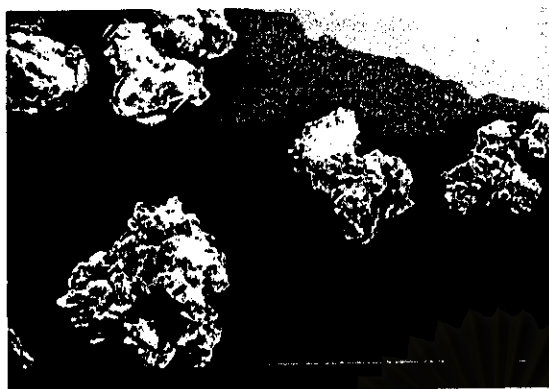


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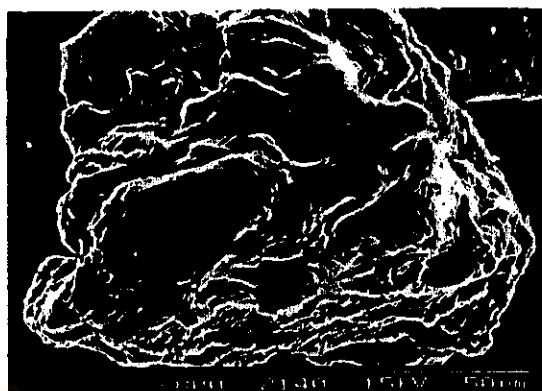


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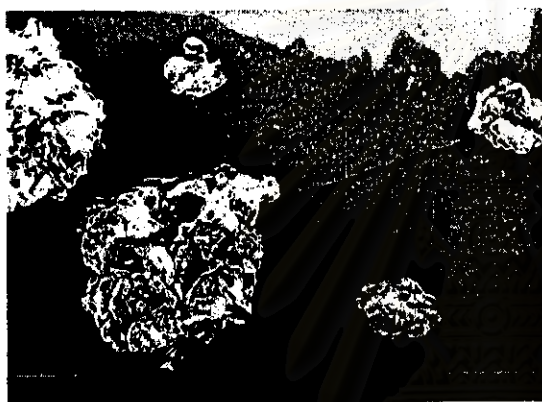
Figure 23 : Scanning electron photomicrographs of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.25%w/v, pectin 5.0%w/v, calcium chloride 1 gm, 2 hrs. hardening time (prep.11,12,13), x60 and x800 magnification  
 (11 = glutaral 0.1 gm, indomethacin 1%  
 12 = glutaral 0.1 gm, indomethacin 2%  
 13 = glutaral 0.25 gm, indomethacin 1%)



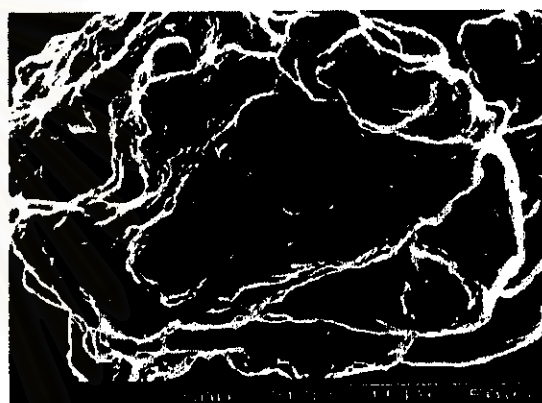
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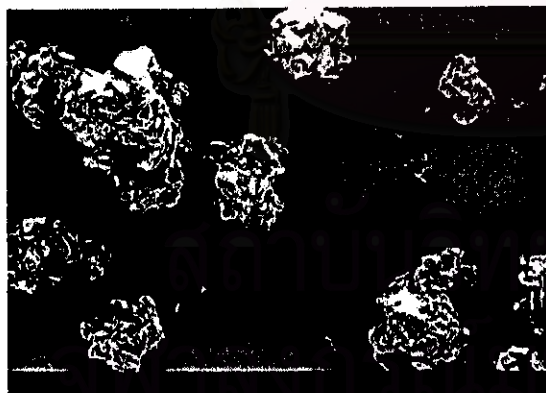
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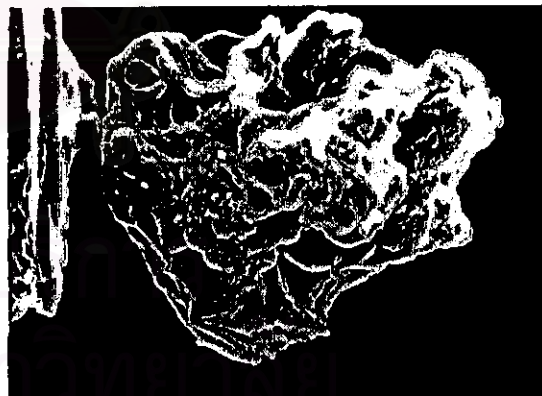
16



16

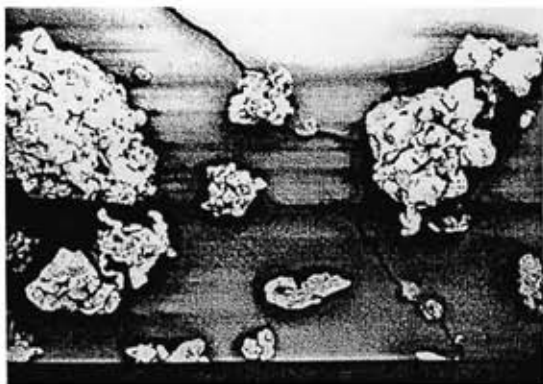


17



17

**Figure 24 : Scanning electron photomicrographs of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.25%w/v, pectin 5.0%w/v, calcium chloride 3 gm, 2 hrs. hardening time (prep.15,16,17), x60 and x800 magnification (15 = glutaral 0.1 gm, indomethacin 1%  
16 = glutaral 0.25 gm, indomethacin 1%  
17 = glutaral 0.25 gm, indomethacin 2%)**



18



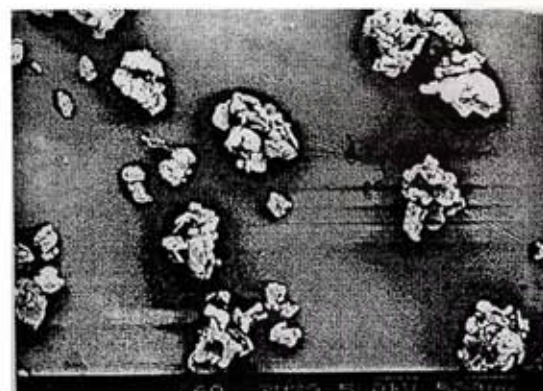
18



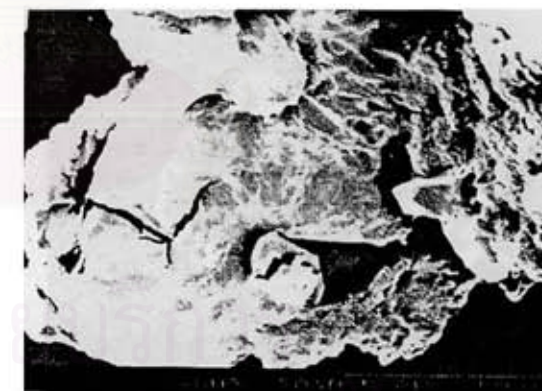
19



19

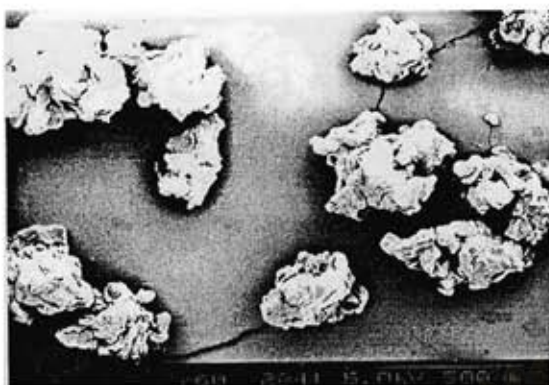


20



20

Figure 25 : Scanning electron photomicrographs of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.25%w/v, pectin 7.5%w/v, calcium chloride 1 gm, 2 hrs. hardening time (prep.18,19,20), x60 and x800 magnification  
 (18 = glutaral 0.1 gm, indomethacin 1%  
 19 = glutaral 0.1 gm, indomethacin 2%  
 20 = glutaral 0.25 gm, indomethacin 1%)



21



21



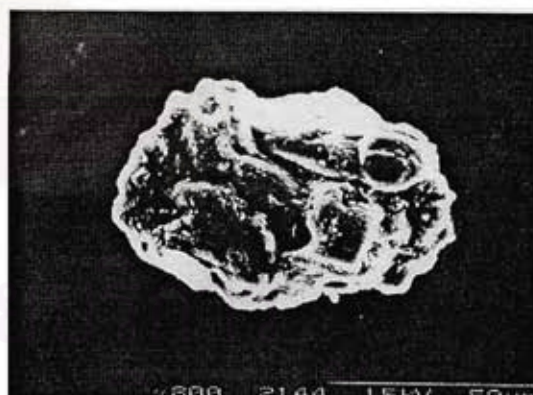
22



22

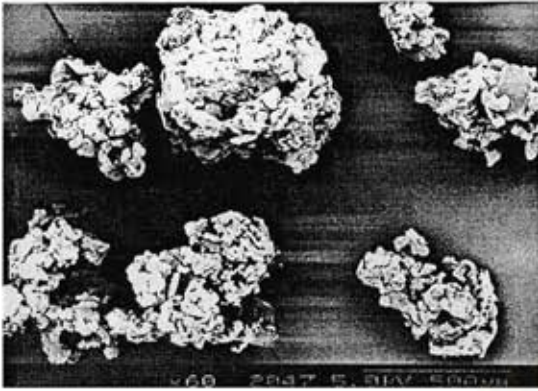


23

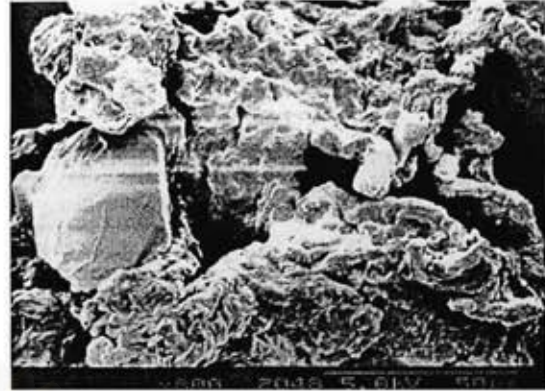


23

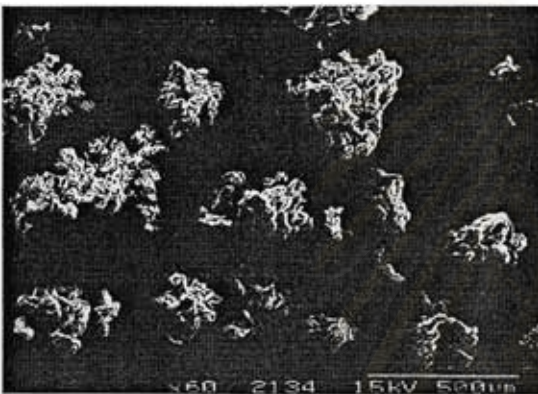
**Figure 26 : Scanning electron photomicrographs of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.25%w/v, pectin 7.5 %w/v, calcium chloride 3 gm, 2 hrs. hardening time (prep.21,22,23), x60 and x800 magnification (21 = glutaral 0.1 gm, indomethacin 1%  
22 = glutaral 0.25 gm, indomethacin 1%  
23 = glutaral 0.25 gm, indomethacin 2%)**



26



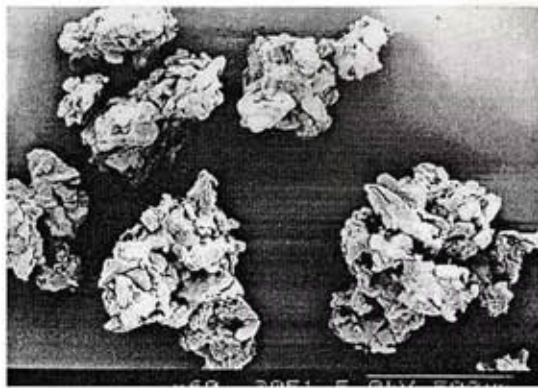
26



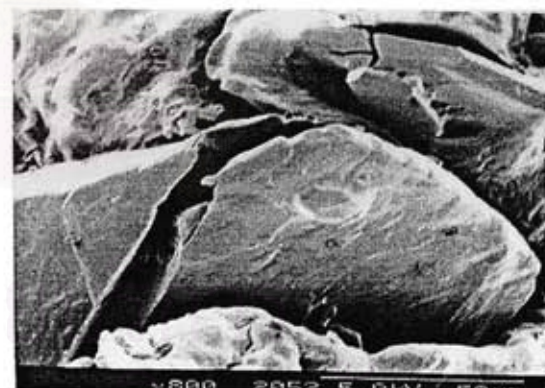
27



27

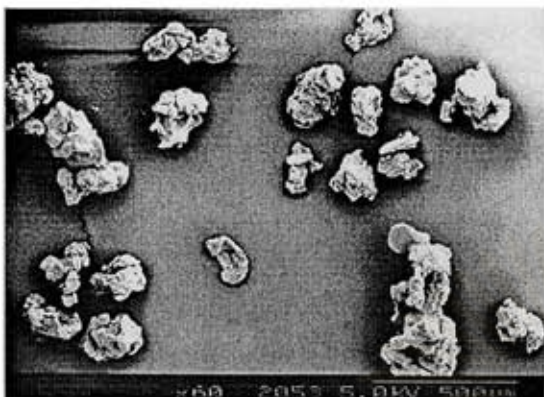


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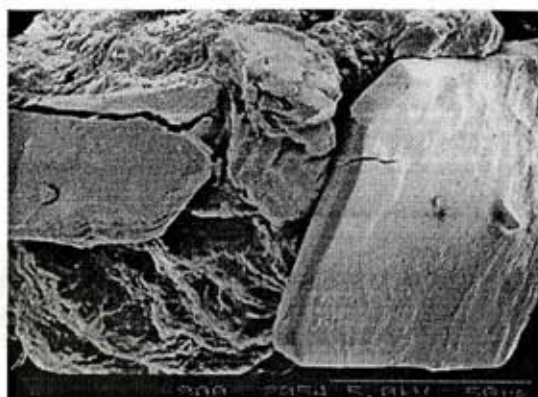


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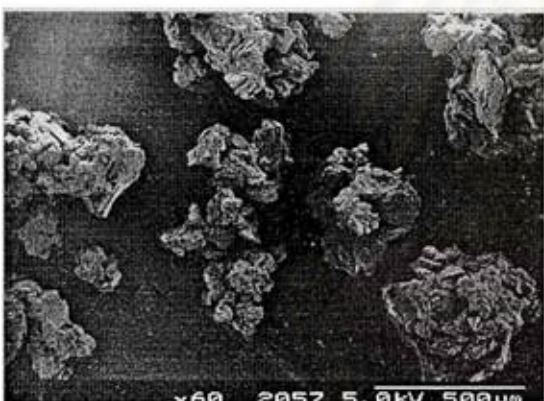
**Figure 27 :** Scanning electron photomicrographs of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.5%w/v, pectin 5.0%w/v, indomethacin 1%, 2 hrs. hardening time (prep.26,27,28), x680 and x800 magnification  
 (26 = calcium chloride 1 gm, glutaral 0.1 gm  
 27= calcium chloride 1 gm, glutaral 0.25 gm  
 28 = calcium chloride 3 gm, glutaral 0.1 gm)



30



30



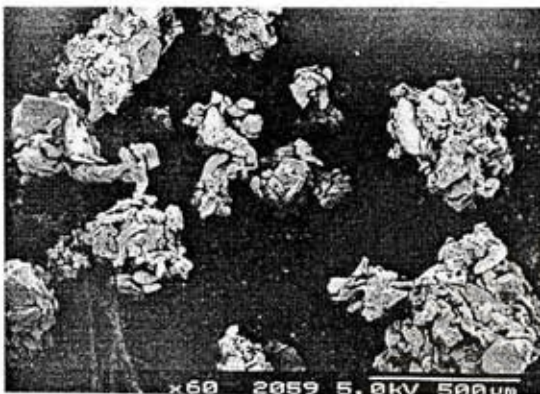
34



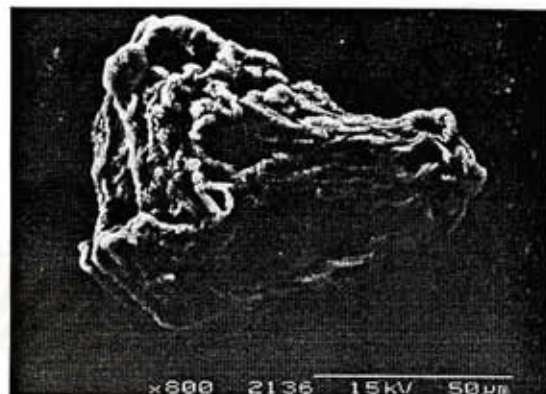
34

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**Figure 28 : Scanning electron photomicrographs of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.5%w/v, pectin 7.5%w/v, indomethacin 1%, 2 hrs. hardening time (prep.30,34), x60 and x800 magnification (30 = calcium chloride 1 gm, glutaral 0.1 gm 34 = calcium chloride 3 gm, glutaral 0.1 gm)**



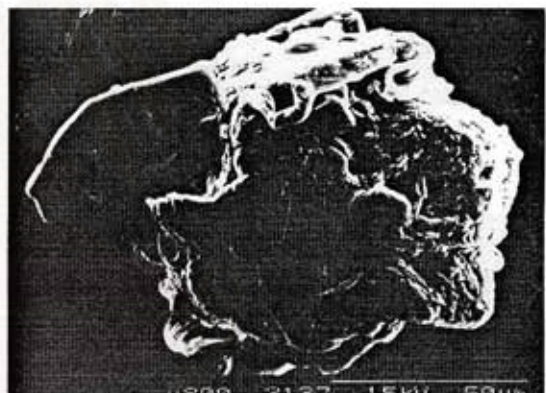
35



35



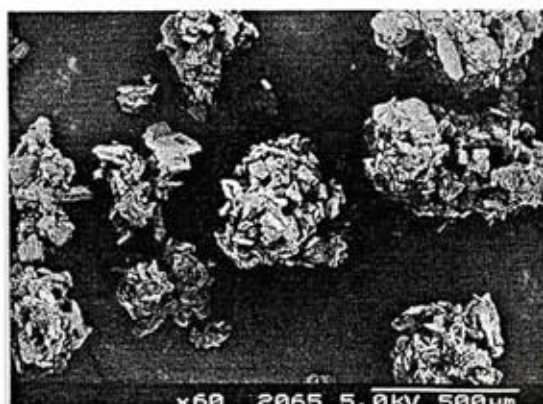
36



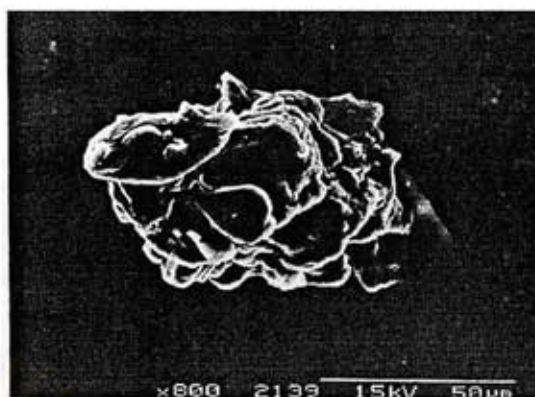
36

Figure 29 : Scanning electron photomicrographs of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.5%w/v, pectin 7.5%w/v, calcium chloride 3 gm, glutaral 0.1 gm, 2 hrs. hardening time (prep.35,36), x60 and x800 magnification (35 = indomethacin 2%  
36 = indomethacin 3%)





14



14



32



32



33



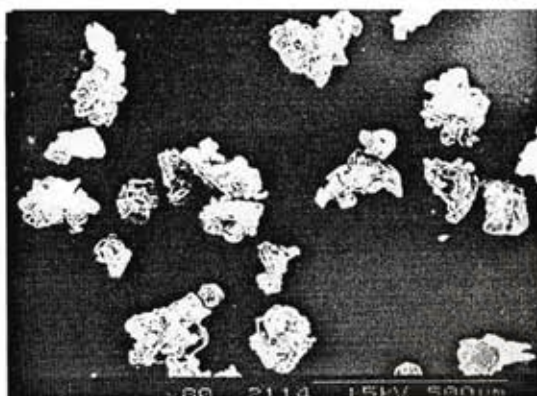
33

Figure 30 : Scanning electron photomicrographs of chitosan-pectin indomethacin microcapsules prepared from calcium chloride 3 gm, no glutaral (prep.14,32,33), x60 and x800 magnification

(14 = chitosan 0.25%w/v, pectin 5.0%w/v, indomethacin 3%)

32 = chitosan 0.5%w/v, pectin 7.5%w/v, indomethacin 1%

33 = chitosan 0.5%w/v, pectin 7.5%w/v, indomethacin 5%)



39



39



41

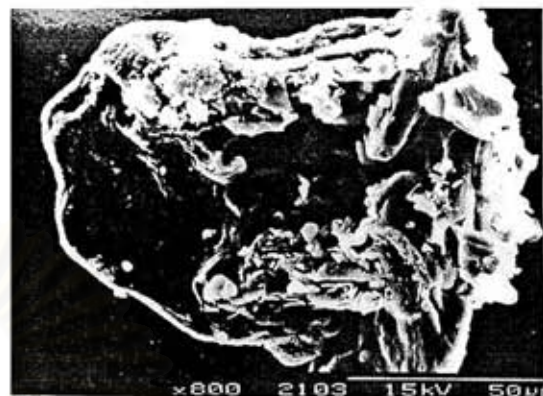


41

Figure 31 : Scanning electron photomicrographs of chitosan-pectin indomethacin microcapsules prepared from calcium chloride 3 gm, no glutaral, indomethacin 3%, (prep.39,41), x80 and x800 magnification  
 (39 = chitosan 0.5%w/v, pectin 10.0%w/v  
 41 = chitosan 0.75%w/v, pectin 10.0%w/v)



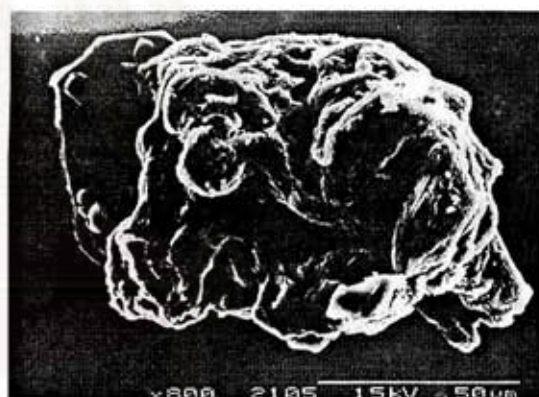
24c



24c



24d

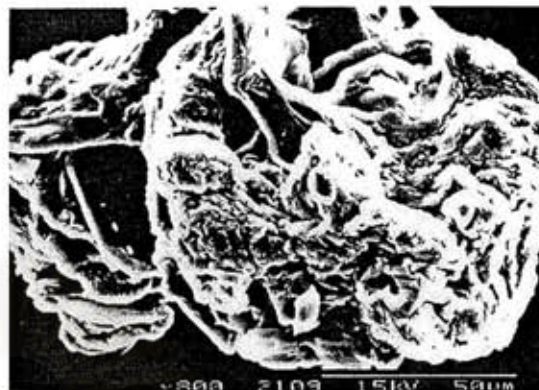


24d

Figure 32 : Scanning electron photomicrographs of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.25%w/v, pectin 7.5%w/v, calcium chloride 3 gm, glutaral 0.25 gm, indomethacin 3% (prep.24c,24d), x80 and x800 magnification (24c = 6 hrs. hardening time  
24d = 18 hrs. hardening time)



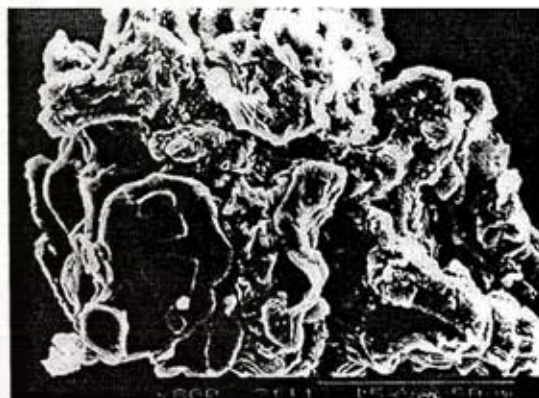
38c



38c



38d



38d

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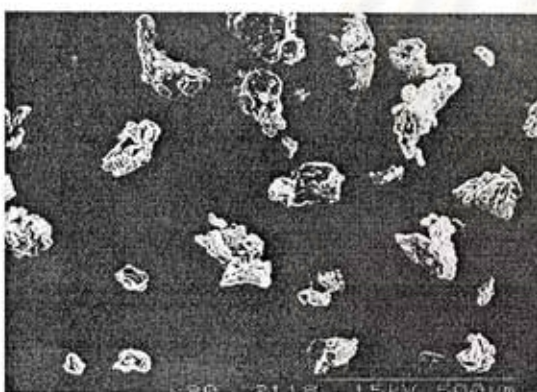
**Figure 33 : Scanning electron photomicrographs of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.5%w/v, pectin 7.5%w/v, calcium chloride 3 gm, glutaral 0.25 gm, indomethacin 3% (prep.38c,38d), x80 and x800 magnification (38c = 6 hrs. hardening time  
38d = 18 hrs. hardening time)**



40c



40c



40d



40d

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Figure 34 : Scanning electron photomicrographs of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.75%w/v, pectin 10.0%w/v, calcium chloride 3 gm, glutaral 0.25 gm, indomethacin 3% (prep.40c,40d), x80 and x800 magnification (40c = 6 hrs. hardening time  
40d = 18 hrs. hardening time)



25d



25d



42c



42c

Figure 35 : Scanning electron photomicrographs of chitosan-pectin indomethacin microcapsules prepared from pectin 10.0%w/v, calcium chloride 3 gm, indomethacin 3%, (prep.25d,42c), x80 and x800 magnification  
 (25d = chitosan 0.25%w/v, glutaral 0.25 gm, 18 hrs. hardening time  
 42c = chitosan 0.75%w/v, glutaral 0.1 gm, 6 hrs. hardening time)

microcapsules. But other topographs of preparations shown in Figures 23, 25, 27 Prep. 26-27, 28 Prep. 30 prepared from using  $\text{CaCl}_2$  1 gm /gm chitosan could be observed many large particles and seemed like solid dispersion.

## 2.2 Size and size distribution

The number of size distribution, percentage frequency and the cumulative percentage frequency undersize are shown in Table 19 in the Appendix. And geometric mean diameter at  $D_{50}$  of microcapsules are displayed in Table 12. The particle size distributions of chitosan-CMC and chitosan-pectin indomethacin microcapsules varied in the range of 40-291 and 40-459  $\mu\text{m}$ , respectively. The particle size distribution of chitosan-CMC was noticeably narrower than chitosan-pectin indomethacin microcapsules. The ranges of 45.71-107.15  $\mu\text{m}$  and 104.71-281.84  $\mu\text{m}$  were geometric mean diameter ( $D_{50}$ ) of chitosan-CMC and chitosan-pectin indomethacin microcapsules, respectively. The minimum and maximum geometric mean diameters of chitosan-CMC indomethacin microcapsules were found in preparation 8a prepared from chitosan 0.5 %w/v, CMC 1.0 %w/v, glutaraldehyde 0.25 gm and preparation 3 prepared from chitosan 0.25 %w/v, CMC 1.5 %w/v, glutaraldehyde 0.25 gm, respectively. In chitosan-pectin indomethacin microcapsules, preparation 24c prepared from chitosan 0.25 %w/v, pectin 7.5 %w/v,  $\text{CaCl}_2$  3 gm, glutaraldehyde 0.25 gm, indomethacin 3 %, 6 hours hardening time and preparation 16 prepared from chitosan 0.25 %w/v, pectin 5.0 %w/v,  $\text{CaCl}_2$  3 gm, glutaraldehyde 0.25 gm, indomethacin 1 %, 2 hours hardening time had minimum and maximum geometric mean diameters, respectively.

a) Effect of chitosan concentration : From Table 12, preparation of chitosan-CMC indomethacin microcapsule with difference of chitosan concentrations

**Table 12 : Geometric mean diameters (D 50) of indomethacin microcapsules prepared from various preparations**

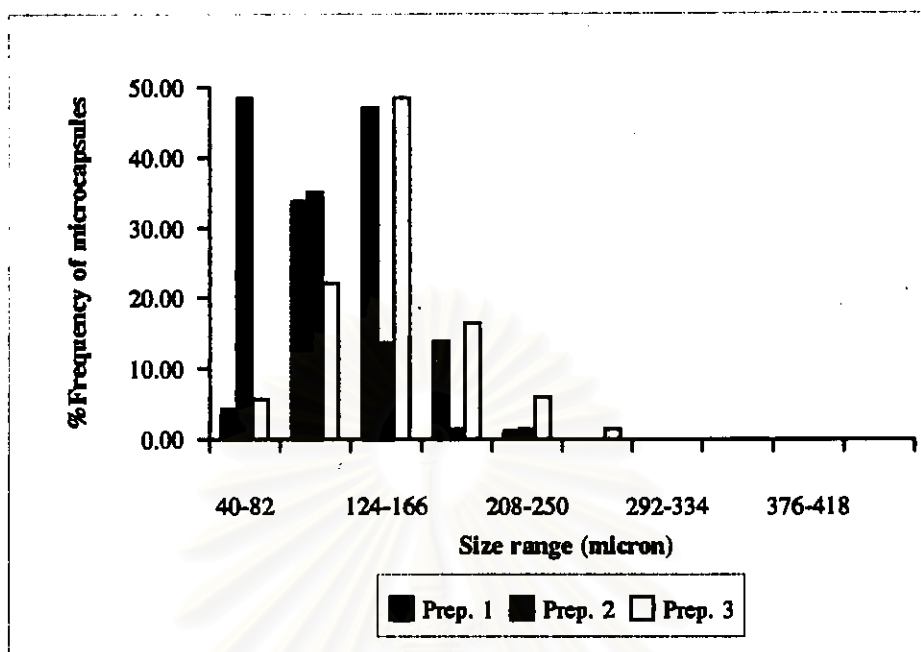
Preparation	D 50 (micron)	Preparation	D 50 (micron)
1	107.15	22	218.78
2	52.48	23	208.93
3	114.28	24c	104.71
4	75.86	24d	114.82
5	85.11	25d	125.89
6	72.44	26	199.53
7	77.62	27	208.93
8a	45.71	28	234.42
9	81.28	30	186.21
10	79.43	32	186.21
11	144.54	33	125.89
12	234.42	34	208.93
13	147.91	35	199.53
14	154.88	36	181.97
15	229.09	38c	120.23
16	281.84	38d	109.65
17	239.88	39	131.83
18	165.96	40c	134.90
19	239.88	40d	114.82
20	158.49	41	109.65
21	234.42	42c	123.03

of 0.25 % and 0.5 %w/v revealed that  $D_{50}$  value increased with the decreasing of chitosan concentration, except  $D_{50}$  value of preparation 4&9 prepared from chitosan 0.35 % and 0.5 %w/v, CMC 1 %w/v, glutaraldehyde 0.25 gm, indomethacin 1.5 % which decreased with the increasing of chitosan concentration. Figures 36-38 illustrate size distribution of chitosan-CMC indomethacin microcapsule various

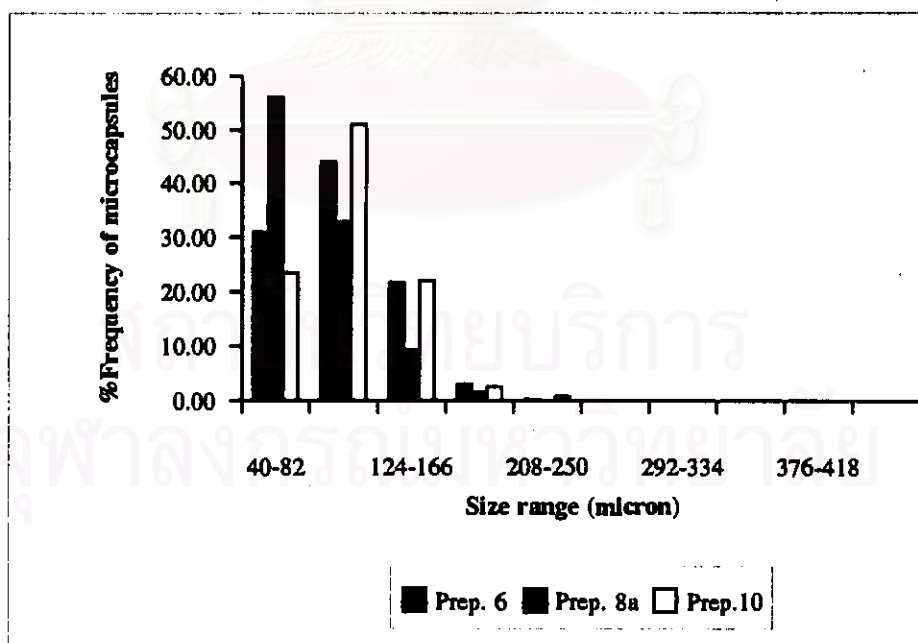


chitosan concentrations. Preparations 4 and 8a displayed the narrowest size distribution. Upon observation of  $D_{50}$  values of chitosan-pectin indomethacin microcapsules, increasing of chitosan concentration increased diameter in preparations 11&26, 13&27, 15&28, 18&30, and 24c&38c. On the contrary,  $D_{50}$  values of preparations 21&34, 24d&38d, and 39&41, decreased with increasing of chitosan concentration. Figures 39-44 show effect of chitosan concentration on size distribution of chitosan-pectin indomethacin microcapsules. The narrow size distribution could be found in Figures 42-44 and while the wider size distribution could be seen in Figures 39-41.

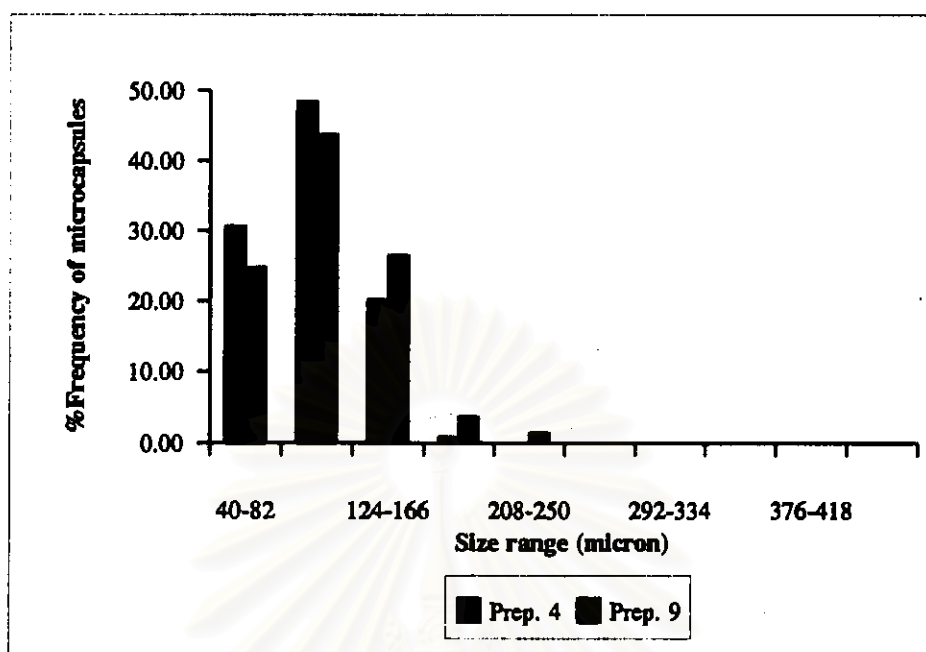
b) Effect of CMC or pectin concentration : Geometric mean diameters of preparations prepared from chitosan 0.25 %w/v, glutaraldehyde 0.25 gm, and various CMC concentrations from 0.75-1.50 %w/v could show that CMC concentration 1 %w/v had the lowest  $D_{50}$  value. The result of  $D_{50}$  value was the same as preparations prepared from chitosan 0.5 %w/v, glutaraldehyde 0.25 gm. Size distributions of chitosan-CMC indomethacin microcapsules prepared from various CMC concentrations shown in Figures 36-37 and 45 Prep.5,7 were of similar pattern in skewed narrow distribution. In the case of chitosan-pectin indomethacin microcapsules,  $D_{50}$  values decreased with increasing of pectin concentration in preparations 16&22, 17&23, 26&30, and 28&34. However, the preparations 11&18, 12&19, 13&20, 15&21, 24d&25d, 38c&40c, and 38d&40d had small diameters with decreasing pectin concentration. The size distributions of chitosan-pectin indomethacin microcapsules various pectin concentrations are shown in Figures 39-43 and 46-47. Figure 42 displays skewed narrow size distribution of preparation prepared from chitosan 0.25 % and 0.5 %w/v, pectin 7.5 % and 10.0 %w/v,  $\text{CaCl}_2$  3 gm, glutaraldehyde 0.25 gm, indomethacin 3 %, 6 hours hardening time. The narrow size distribution had the same pattern in preparations prepared



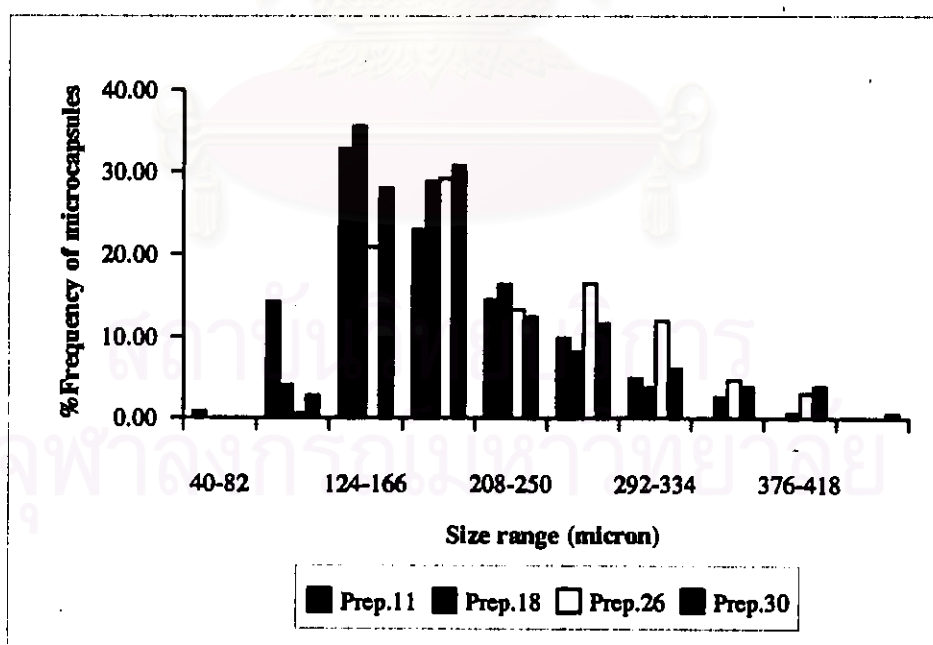
**Figure 36 :** Size distributions of chitosan-CMC indomethacin microcapsules prepared from chitosan 0.25%w/v, glutaral 0.25 gm, indomethacin 1%, 2 hrs.hardening time (Prep.1,2,3)  
 (Prep. 1 = CMC 0.75%w/v, Prep. 2 = CMC 1%w/v, Prep. 3 = CMC 1.5%w/v)



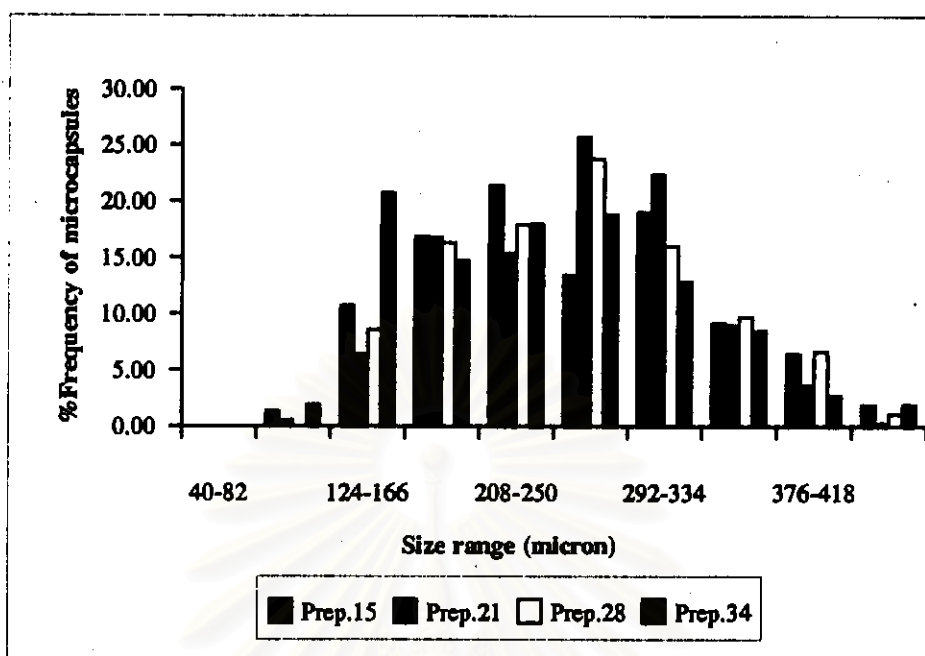
**Figure 37 :** Size distributions of chitosan-CMC indomethacin microcapsules prepared from chitosan 0.5%w/v, glutaral 0.25 gm, indomethacin 1%, 2 hrs.hardening time (Prep.6,8a,10)  
 (Prep. 6 = CMC 0.75%w/v, Prep. 8a = CMC 1%w/v, Prep. 10 = CMC 1.5%w/v)



**Figure 38 :** Size distributions of chitosan-CMC indomethacin microcapsules prepared from CMC 1% w/v, glutaral 0.25 gm, indomethacin 1.5%, 2 hrs. hardening time (Prep. 4, 9)  
(Prep. 4 = chitosan 0.35% w/v, Prep. 9 = chitosan 0.5% w/v)



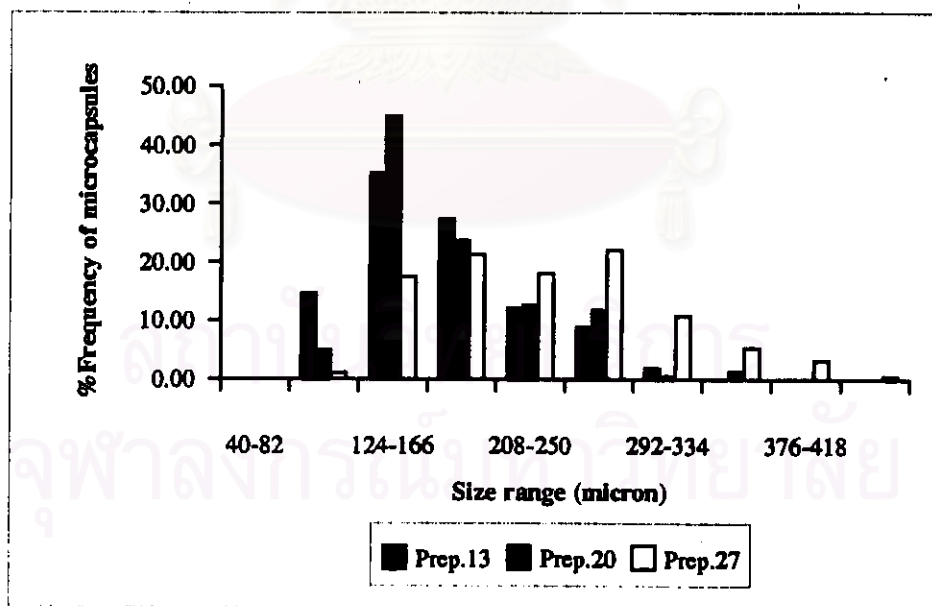
**Figure 39 :** Size distributions of chitosan-pectin indomethacin microcapsules prepared from calcium chloride 1 gm, glutaral 0.1 gm, indomethacin 1%, 2 hrs. hardening time (Prep. 11, 18, 26, 30)  
(Prep. 11 and 18 = chitosan 0.25% w/v, pectin 5% and 7.5% w/v  
Prep. 26 and 30 = chitosan 0.5% w/v, pectin 5% and 7.5% w/v)



**Figure 40 :** Size distributions of chitosan-pectin indomethacin microcapsules prepared from calcium chloride 3 gm, glutaral 0.1 gm, indomethacin 1%, 2 hrs. hardening time (Prep.15,21,28,34)

(Prep. 15 and 21 = chitosan 0.25% w/v, pectin 5% and 7.5% w/v

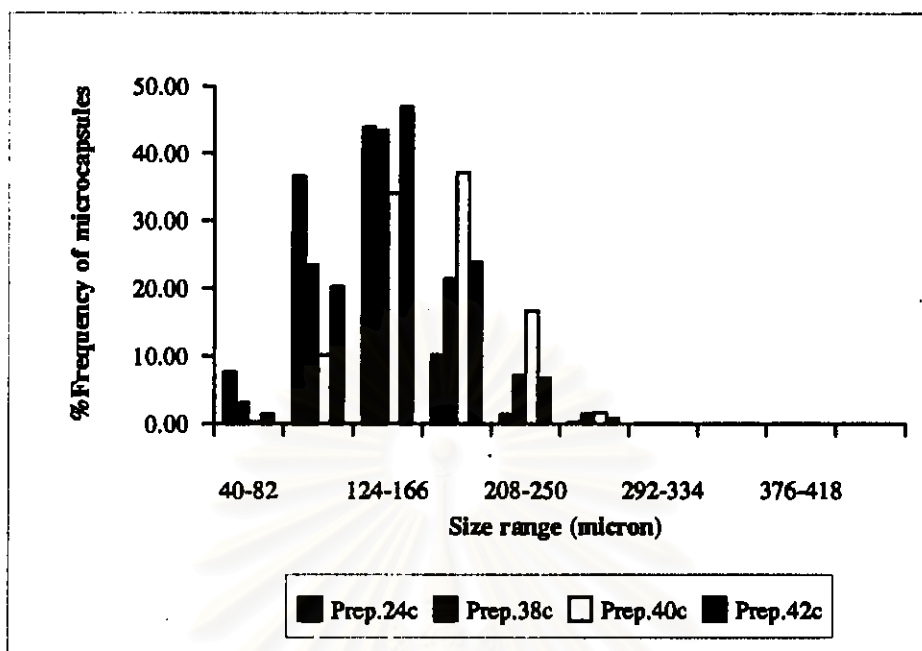
Prep. 28 and 34 = chitosan 0.5% w/v, pectin 5% and 7.5% w/v)



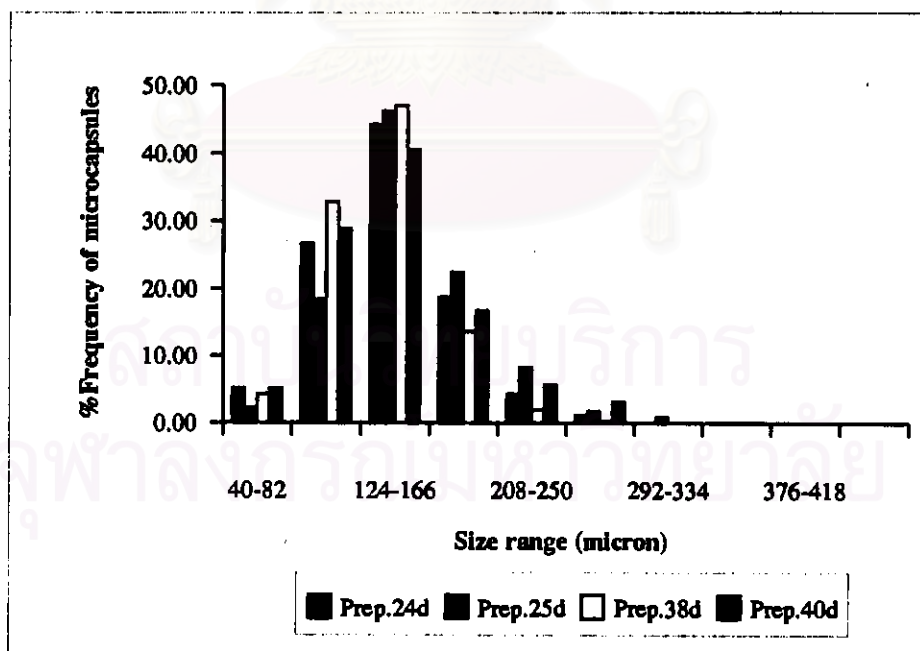
**Figure 41 :** Size distributions of chitosan-pectin indomethacin microcapsules prepared from calcium chloride 1 gm, glutaral 0.25 gm, indomethacin 1%, 2 hrs. hardening time (Prep.13,20,27)

(Prep. 13 and 20 = chitosan 0.25% w/v, pectin 5% and 7.5% w/v

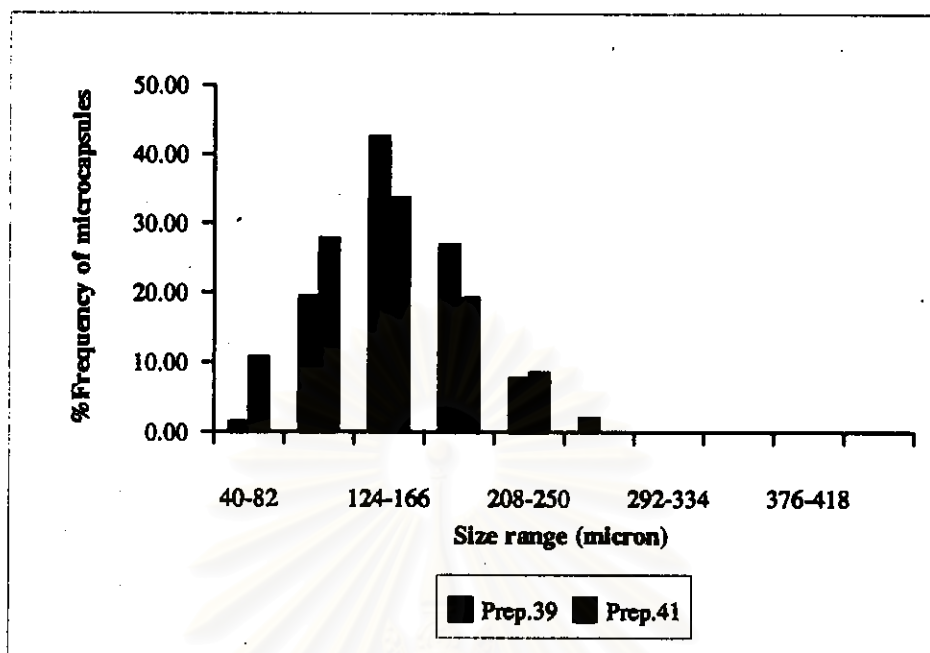
Prep. 27 = chitosan 0.5% w/v, pectin 5% w/v)



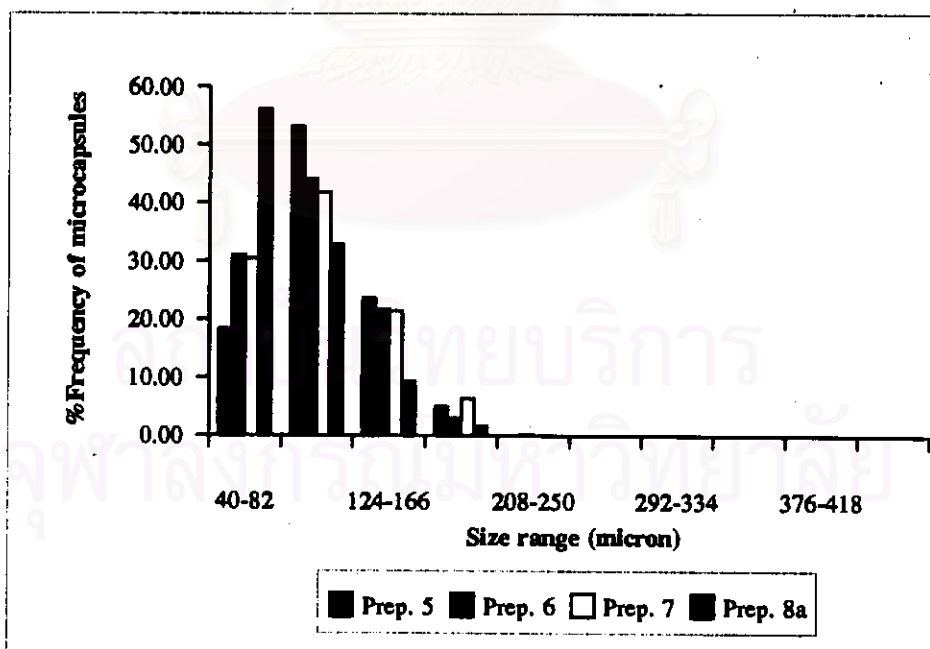
**Figure 42 :** Size distributions of chitosan-pectin indomethacin microcapsules prepared from calcium chloride 3 gm, indomethacin 3%, 6 hrs. hardening time (Prep. 24c, 38c, 40c, 42c)  
 (Prep. 24c = chitosan 0.25% w/v, pectin 7.5% w/v, glutaral 0.25 gm  
 Prep. 38c and 40c = chitosan 0.5% w/v, pectin 7.5% and 10% w/v, glutaral 0.25 gm  
 Prep. 42c = chitosan 0.5% w/v, pectin 10% w/v, glutaral 0.1 gm)



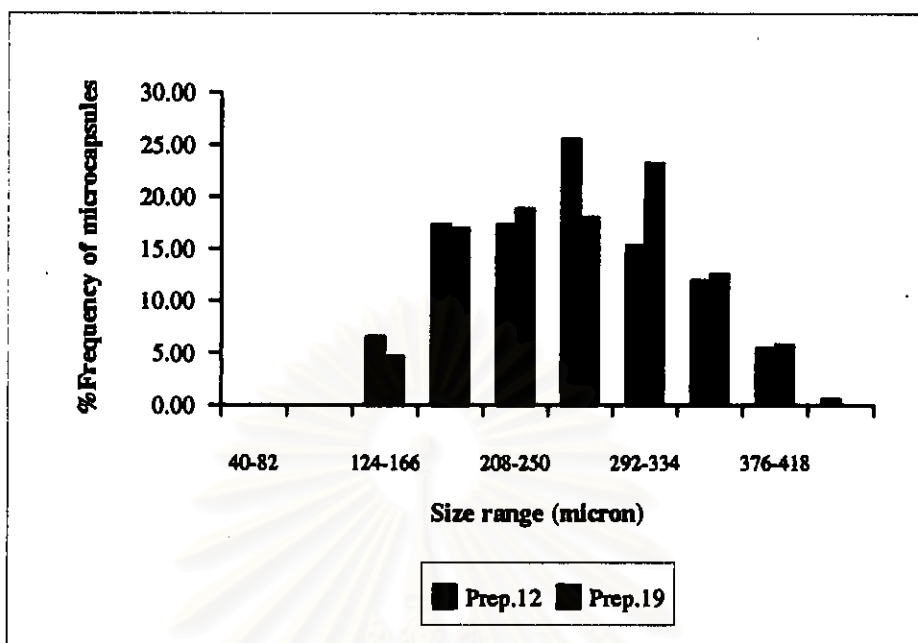
**Figure 43 :** Size distributions of chitosan-pectin indomethacin microcapsules prepared from calcium chloride 3 gm, glutaral 0.25 gm, indomethacin 3%, 18 hrs. hardening time (Prep. 24d, 25d, 38d, 40d)  
 (Prep. 24d and 25d = chitosan 0.25% w/v, pectin 7.5% and 10% w/v  
 Prep. 38d and 40d = chitosan 0.5% w/v, pectin 7.5% and 10% w/v)



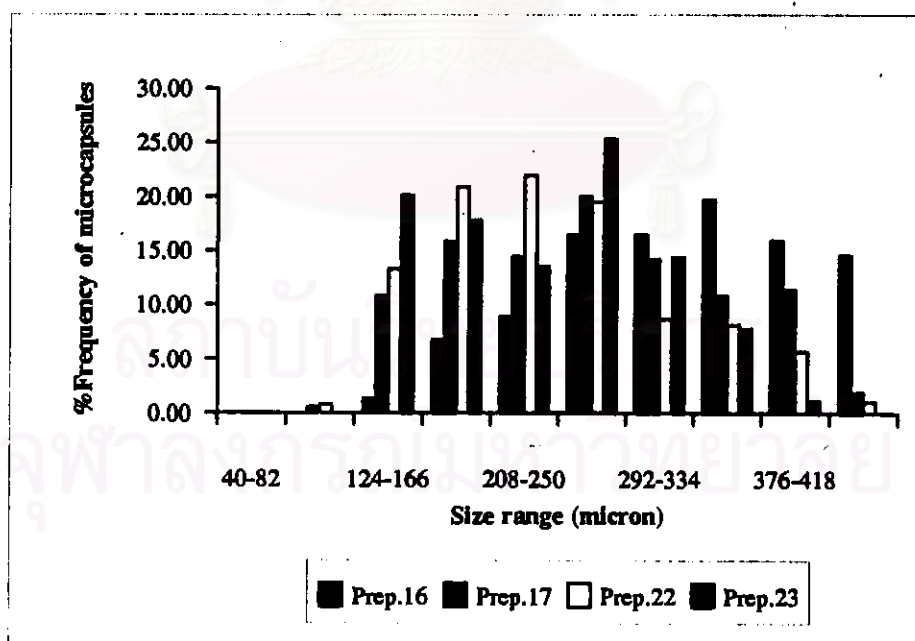
**Figure 44 :** Size distributions of chitosan-pectin indomethacin microcapsules prepared from pectin 10%w/v, calcium chloride 3 gm, no glutaral , indomethacin 3% (Prep.39,41)  
(Prep. 39 = chitosan 0.5%w/v, Prep. 41 = chitosan 0.75 %w/v)



**Figure 45 :** Size distributions of chitosan-CMC indomethacin microcapsules prepared from chitosan 0.5%w/v, indomethacin 1%, 2 hrs. hardening time (Prep.5,6,7,8a)  
(Prep. 5 and 6 = CMC 0.75%w/v, glutaral 0.1 and 0.25 gm  
Prep. 7 and 8a = CMC 1%w/v, glutaral 0.1 and 0.25 gm)



**Figure 46 :** Size distributions of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.25%w/v, calcium chloride 1 gm, glutaral 0.1 gm, indomethacin 2%, 2 hrs. hardening time (Prep.12,19)  
(Prep. 12 = pectin 5%w/v, Prep. 19 = pectin 7.5%w/v)



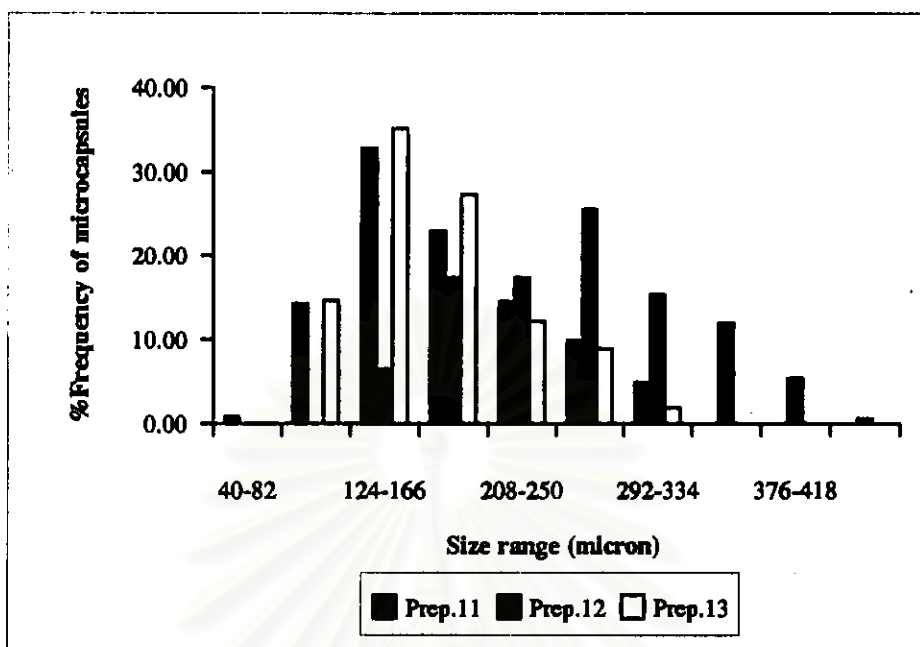
**Figure 47 :** Size distributions of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.25%w/v, calcium chloride 3 gm, glutaral 0.25 gm, 2 hrs. hardening time (Prep.16,17,22,23)  
(Prep. 16 and 17 = pectin 5%w/v, indomethacin 1% and 2%  
Prep. 22 and 23 = pectin 7.5%w/v, indomethacin 1% and 2%)

from the same conditions with 18 hours hardening time shown in Figure 43.

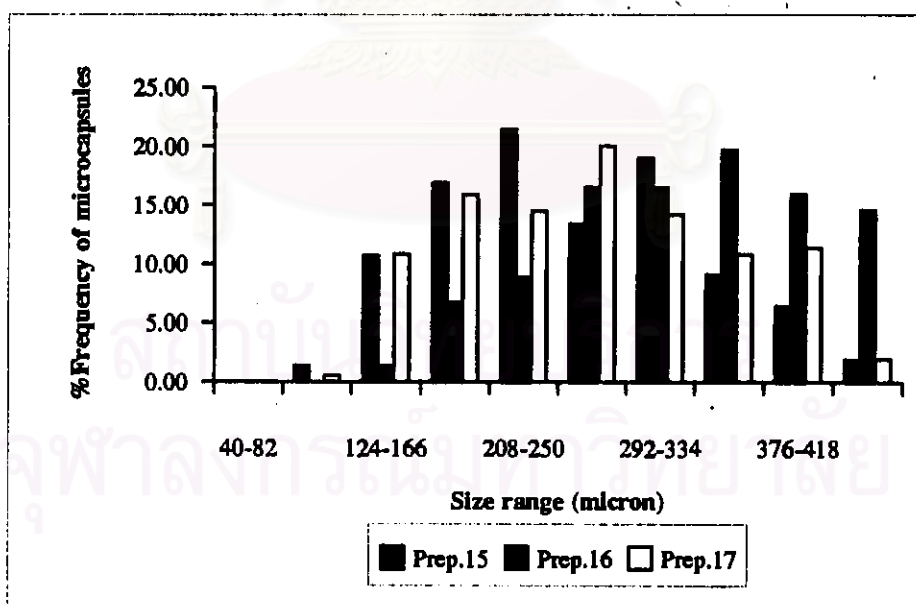
c) Effect of glutaraldehyde content : From the results in Table 12, for the preparations of indomethacin microcapsules prepared from chitosan-CMC with difference of glutaraldehyde content of 0.1 and 0.25 gm /gm polymer,  $D_{50}$  value decreased with increasing of glutaraldehyde content. Figure 45 shows similar pattern of size distribution of chitosan-CMC indomethacin microcapsule prepared from various glutaraldehyde contents. The effect of glutaraldehyde content on  $D_{50}$  values of chitosan-pectin indomethacin microcapsules was that lower diameter was found at higher glutaraldehyde content in preparations 18&20 and 21&22. On the contrary,  $D_{50}$  values of preparations 11&13, 15&16, 26&27, and 32&34 increased with increasing of glutaraldehyde content. Figures 48-53 show effect of glutaraldehyde content on size distributions of chitosan-pectin indomethacin microcapsules.

d) Effect of indomethacin content : From Table 12, among the preparations of indomethacin microcapsules prepared by chitosan-CMC with difference in indomethacin contents of 1% and 1.5 %,  $D_{50}$  values of the preparation using indomethacin 1 % was less than that of the preparation using 1.5 %. Figure 54 shows that size distribution of preparation 8a was narrower than that of preparation 9. In the comparison of the geometric mean diameters of preparations 11&12, 18&19 using  $\text{CaCl}_2$  1 gm /gm chitosan,  $D_{50}$  values increased with adding more indomethacin. While among preparations 16&17, 22&23, 32&33, and 34&35&36 using  $\text{CaCl}_2$  3 gm,  $D_{50}$  values decreased with increasing of indomethacin in the process. Figures 48-51, 53 and 55 show effect of indomethacin content on size distributions of chitosan-pectin indomethacin microcapsules. Size distribution of preparation 11 in Figure 48 was narrower than preparation 12.

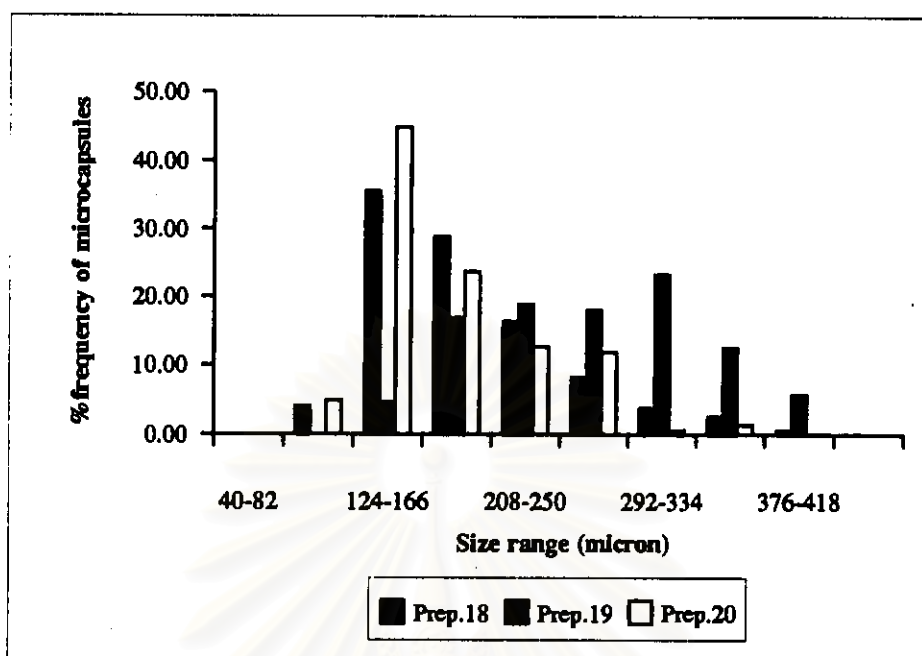




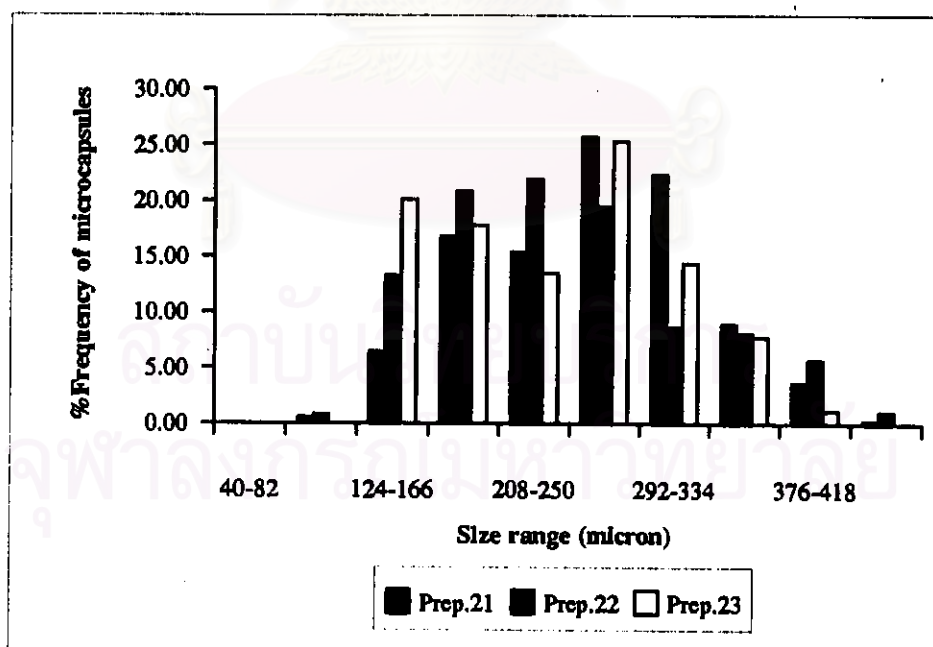
**Figure 48 :** Size distributions of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.25 %w/v, pectin 5%w/v, calcium chloride 1 gm, 2 hrs. hardening time (Prep.11,12,13)  
 (Prep. 11 and 12 = glutaral 0.1 gm, indomethacin 1% and 2%  
 Prep. 13 = glutaral 0.25 gm, indomethacin 1%)



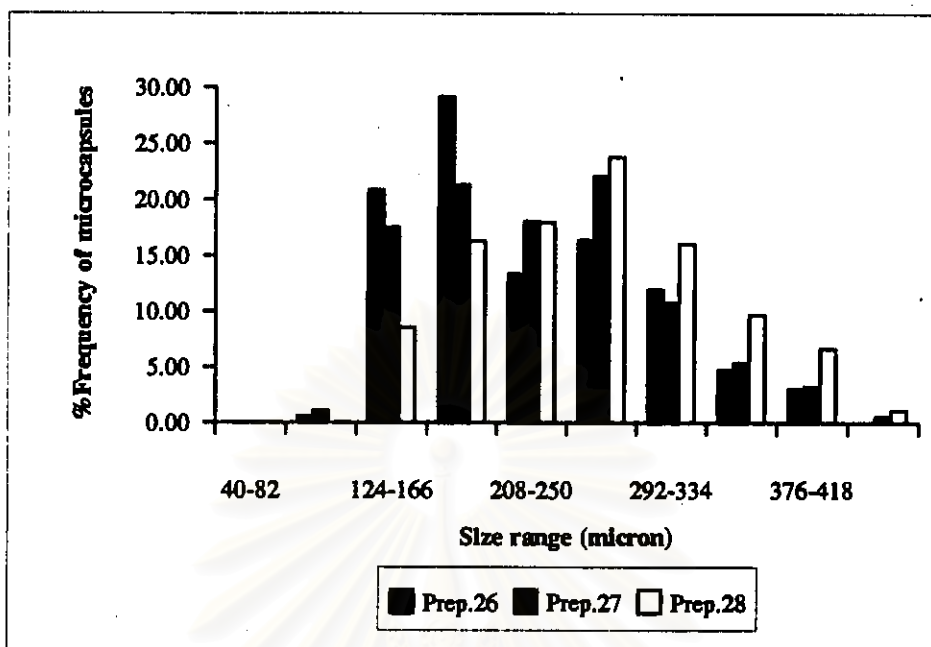
**Figure 49 :** Size distributions of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.25 %w/v, pectin 5%w/v, calcium chloride 3 gm, 2 hrs. hardening time (Prep.15,16,17)  
 (Prep. 15 = glutaral 0.1 gm, indomethacin 1%  
 Prep.16 and 17 = glutaral 0.25 gm, indomethacin 1% and 2%)



**Figure 50 :** Size distributions of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.25% w/v, pectin 7.5% w/v, calcium chloride 1 gm, 2 hrs. hardening time (Prep.18,19,20)  
 (Prep. 18 and 19 = glutaral 0.1 gm, indomethacin 1% and 2 %  
 Prep. 20 = glutaral 0.25 gm, indomethacin 1%)

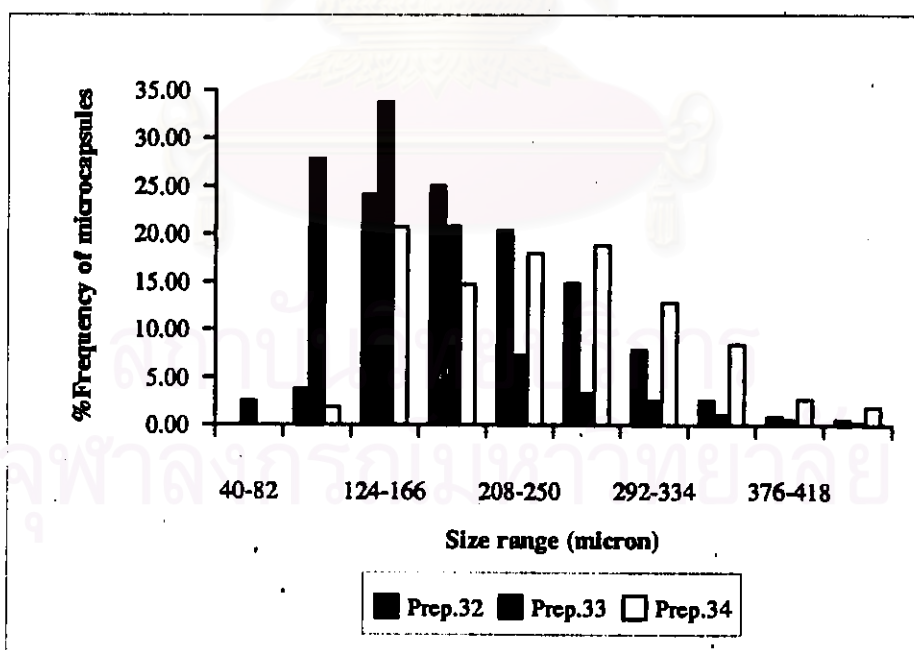


**Figure 51 :** Size distributions of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.25% w/v, pectin 7.5% w/v, calcium chloride 3 gm, 2 hrs. hardening time (Prep.21,22,23)  
 (Prep. 21 = glutaral 0.1 gm, indomethacin 1%  
 Prep. 22 and 23 = glutaral 0.25 gm, indomethacin 1% and 2%)



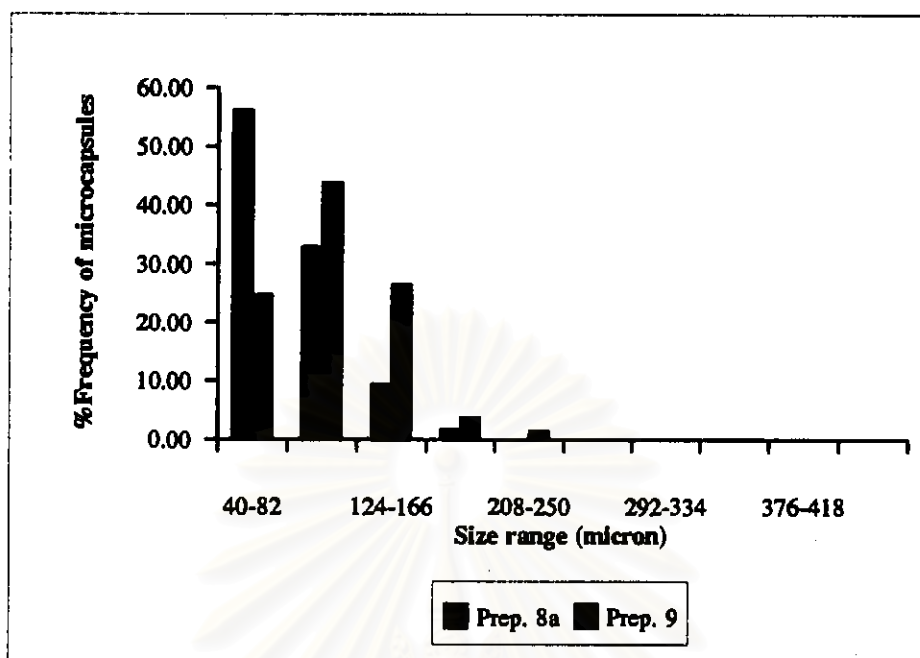
**Figure 52 :** Size distributions of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.5%w/v, pectin 5%w/v, indomethacin 1%, 2hrs. hardening time (Prep.26,27,28)

(Prep. 26 and 27 = calcium chloride 1 gm, glutaral 0.1 gm and 0.25 gm  
Prep. 28 = calcium chloride 3 gm, glutaral 0.1 gm)

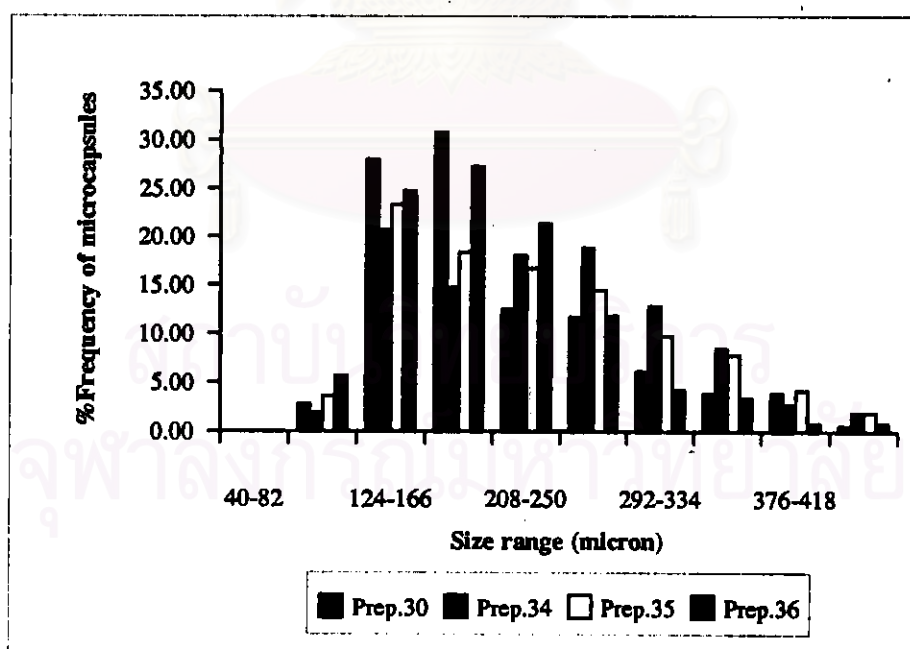


**Figure 53 :** Size distributions of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.5%w/v, pectin 7.5%w/v, calcium chloride 3 gm, 2 hrs. hardening time (Prep.32,33,34)

(Prep. 32 and 33 = no glutaral, indomethacin 1% and 5%  
Prep. 34 = glutaral 0.1 gm, indomethacin 1%)



**Figure 54 :** Size distributions of chitosan-CMC indomethacin microcapsules prepared from chitosan 0.5% w/v, CMC 1% w/v, glutaral 0.25 gm, 2hrs. hardening time (Prep. 8a,9) (Prep. 8a = indomethacin 1%, Prep. 9 = indomethacin 1.5%)



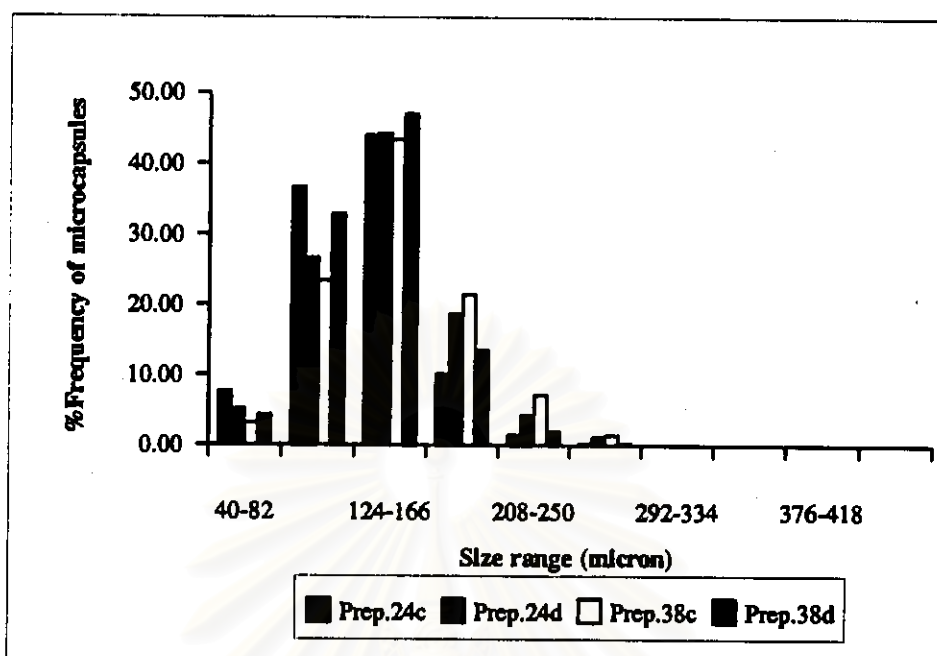
**Figure 55 :** Size distributions of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.5% w/v, pectin 7.5% w/v, glutaral 0.1 gm, 2 hrs. hardening time (Prep. 30,34,35,36) (Prep. 30 = calcium chloride 1 gm, indomethacin 1% (Prep. 34,35,36 = calcium chloride 3 gm, indomethacin 1%, 2%, 3%))

e) Effect of hardening time : Preparations hardened 18 hours had lower  $D_{50}$  value than 6 hours except preparation 24c&24d prepared from chitosan 0.25 %w/v, pectin 7.5 %w/v. Figures 56 and 57 show effect of hardening time on size distribution of chitosan-pectin indomethacin microcapsules. They displayed narrow size distribution in the range of 40-291  $\mu\text{m}$ .

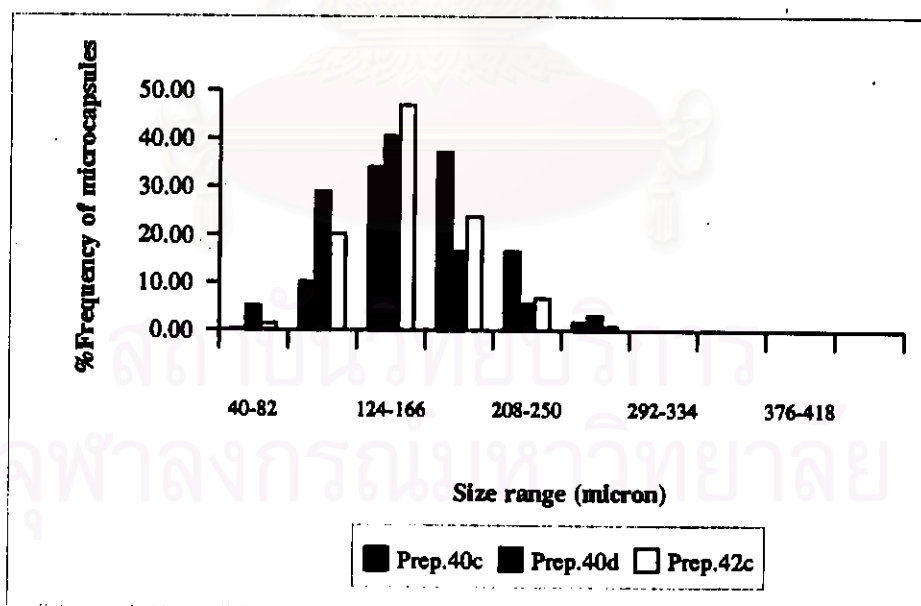
f) Effect of calcium chloride content & From Table 12, among preparations 11&15, 13&16, 18&21, 20&22, 26&28, and 30&34 of chitosan-pectin indomethacin microcapsules with difference of  $\text{CaCl}_2$  contents of 1 and 3 gm /gm chitosan, higher  $\text{CaCl}_2$  content resulted in the larger geometric mean diameter. Figures 52, 55, and 58-59 show effect of  $\text{CaCl}_2$  content on size distributions of chitosan-pectin indomethacin microcapsules. Figure 58 shows size distributions of preparations 11&15 and 13&16 prepared from chitosan 0.25 %w/v, pectin 5.0 % w/v, glutaraldehyde 0.1 gm and 0.25 gm, respectively. The lower  $\text{CaCl}_2$  content gave narrower size distribution, in the range of 40-333  $\mu\text{m}$ . Size distribution of preparations 26&28, 30&34, and 18&21, 20&22 shown in Figures 52, 55, and 59 exhibited skewed distribution in preparation with lower  $\text{CaCl}_2$  content.

### 2.3 Percentage of drug entrapment, drug recovery, and yield

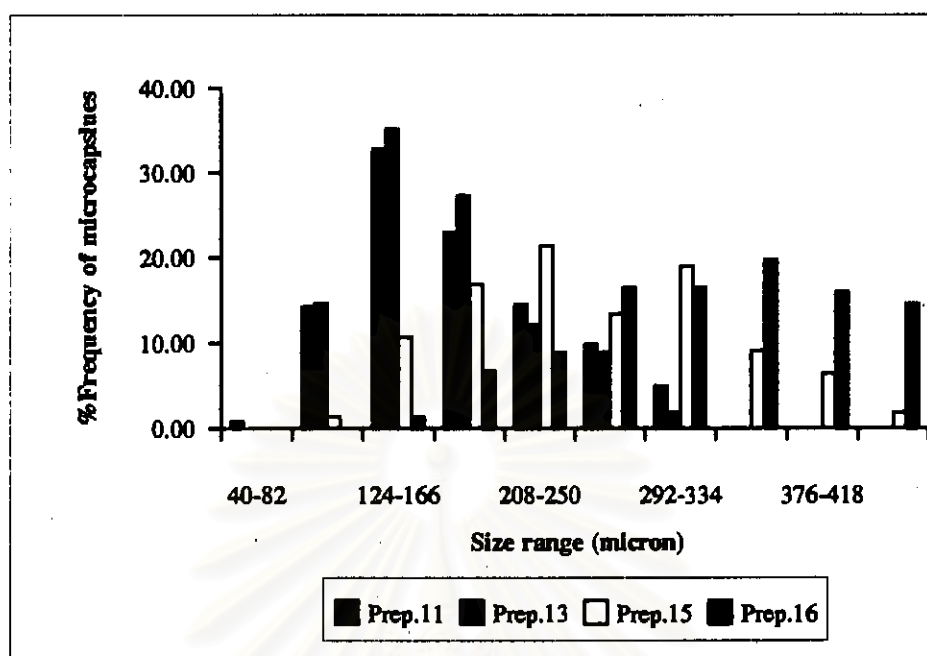
Indomethacin, an insoluble drug, could be entrapped in chitosan-CMC and chitosan-pectin by complex coacervation. Drug entrapments of chitosan-CMC indomethacin microcapsules in various preparations were in the range of 25.81-50.25%. The drug could be recovered in the range of 42.73-91.00%. The percentage of yield ranged 27.89-58.00%. In various preparations of chitosan-pectin microcapsules, drug entrapment, drug recovery, and yield were in the range of 8.50-43.38%, 40.73-99.50%, and 38.28-75.92%, respectively. The results are shown in



**Figure 56 :** Size distributions of chitosan-pectin indomethacin microcapsules prepared from pectin 7.5%, calcium chloride 3 gm, glutaral 0.25 gm, indomethacin 3% (Prep.24c,24d,38c,38d)  
 (Prep. 24c and 24d = chitosan 0.25%w/v, 6 and 18 hrs. hardening time  
 Prep. 38c and 38d = chitosan 0.5%w/v, 6 and 18 hrs. hardening time)



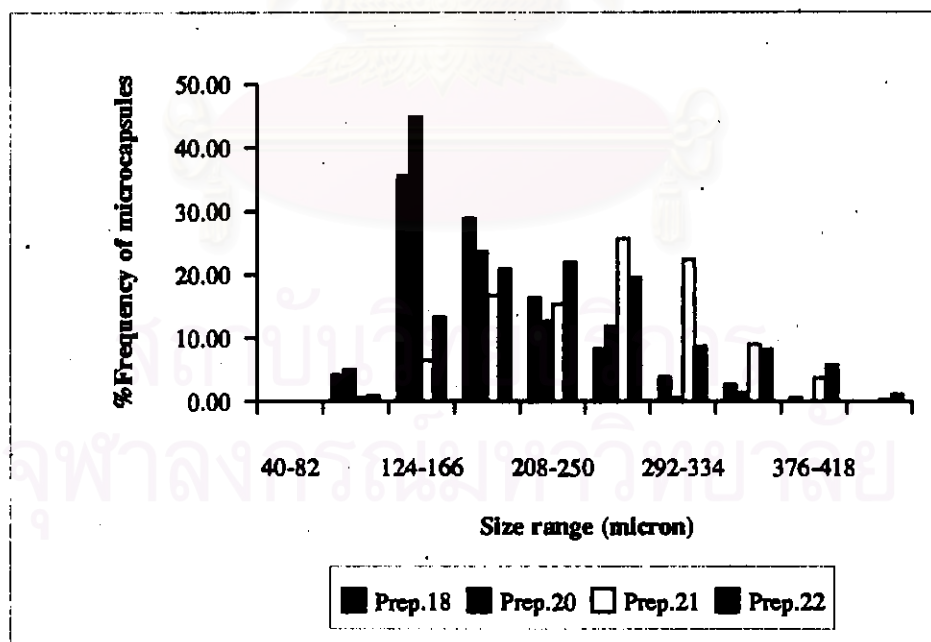
**Figure 57 :** Size distributions of chitosan-pectin indomethacin microcapsules prepared from pectin 10%, calcium chloride 3 gm, glutaral 0.25 gm, indomethacin 3% (Prep.40c,40d,42c)  
 (Prep.40c and 40d=chitosan 0.5%w/v,glutaral 0.25 gm,6 and 18 hrs.hardening time  
 Prep. 42c = chitosan 0.75%w/v, glutaral 0.1 gm, 6 hrs. hardening time)



**Figure 58 :** Size distributions of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.25%w/v, pectin 5%w/v, indomethacin 1%, 2hrs. hardening time (Prep.11,13,15,16)

(Prep. 11 and 13 = calcium chloride 1 gm, glutaral 0.1 gm and 0.25 gm

Prep. 15 and 16 = calcium chloride 3 gm, glutaral 0.1 gm and 0.25 gm)



**Figure 59 :** Size distributions of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.25%w/v, pectin 7.5%w/v, indomethacin 1%, 2 hrs. hardening time (Prep.18,20,21,22)

(Prep. 18 and 20 = calcium chloride 1 gm, glutaral 0.1 gm and 0.25 gm

Prep. 21 and 22 = calcium chloride 3 gm, glutaral 0.1 gm and 0.25 gm)

Tables 13 and 14.

There were many variables such as concentration of chitosan, CMC, pectin, content of glutaraldehyde, content of indomethacin, hardening time, and content of calcium chloride for chitosan-pectin microcapsules. On varying of chitosan concentrations in the chitosan-CMC microcapsule (preparations 1&6, 2&8a, 3&10), higher concentration of chitosan resulted in higher percentage of drug entrapment and drug recovery but lower percentage of yield. While in the chitosan-pectin microcapsule (preparations 11&26, 13&27, 15&28, 18&30, 21&34, 24c&38c, 24d&38d), increasing concentration of chitosan decreased the percentage of drug entrapment, drug recovery and yield. Among various concentrations of CMC, preparations 2 and 8a showed the highest of %drug entrapment and drug recovery, whereas increasing concentration of pectin seemed to decrease of percentage of drug entrapment but increase of percentage of drug recovery. However, percentage of yield increased with increasing of CMC or pectin concentrations.

The percentage of drug entrapment, drug recovery and yield increased when the preparations had more content of glutaraldehyde from 0.1 to 0.25 gm /gm polymer or more loading indomethacin. At hardening time of 2 and 3 hours of chitosan-CMC microcapsules, there was no difference in percentage of drug entrapment, drug recovery and yield, but in the process of chitosan-pectin microcapsules, higher percentage of drug entrapment, drug recovery and yield were obtained when increasing the hardening time from 6 to 18 hours. For chitosan-pectin microencapsulation with calcium chloride in chitosan solution, the percentage of drug entrapment, drug recovery and yield increased when the content of calcium chloride increased from 1 to 3 gm /gm chitosan.



**Table 13 : Percentages of drug entrapment, drug recovery and yield of indomethacin microcapsules of chitosan-carboxymethylcellulose**

<b>Preparation</b>	<b>% Drug Entrapment</b>	<b>% Drug Recovery</b>	<b>% Yield</b>
1	30.52	42.73	36.52
2	35.85	72.15	49.29
3	25.81	68.46	57.90
4	52.30	91.00	58.00
5	30.94	55.41	26.80
6	32.14	53.03	27.89
7	34.12	67.72	30.14
8a	36.86	74.77	36.36
8b	36.93	75.44	36.43
9	50.25	87.13	40.61
10	29.30	85.17	43.63

**Table 14 : Percentages of drug entrapment, drug recovery and yield of indomethacin microcapsules of chitosan-pectin**

<b>Preparation</b>	<b>% Drug Entrapment</b>	<b>% Drug Recovery</b>	<b>% Yield</b>
11	16.30	75.56	57.37
12	27.73	71.85	57.06
13	14.39	72.15	59.78
14	39.33	88.38	71.91
15	11.60	55.95	59.68
16	14.66	68.39	57.66
17	26.51	77.65	64.50

**Table 14 : Percentages of drug entrapment, drug recovery and yield of indomethacin microcapsules of chitosan-pectin (continue)**

<b>Preparation</b>	<b>%Drug Entrapment</b>	<b>%Drug Recovery</b>	<b>%Yield</b>
18	10.67	69.80	61.81
19	17.31	70.13	69.93
20	9.21	68.94	70.69
21	10.48	72.92	65.78
22	9.71	78.03	75.92
23	17.83	85.04	74.10
24c	30.69	80.60	62.61
24d	32.52	89.23	65.42
25d	27.55	88.11	58.73
26	10.47	40.73	38.28
27	12.56	49.36	38.66
28	13.25	64.50	47.88
30	8.50	58.77	54.68
32	8.93	62.28	55.07
33	43.38	85.55	64.16
34	11.82	77.66	59.21
35	22.02	81.86	60.09
36	31.71	91.70	64.27
38c	28.98	72.54	47.86
38d	31.01	75.81	53.51
39	26.71	83.58	47.73
40c	26.70	81.41	46.51
40d	24.22	77.12	48.58
41	35.25	90.19	38.87
42c	37.36	99.50	40.46

## 2.4 Drug release study of indomethacin microcapsules

The drug release data of indomethacin microcapsule prepared by complex coacervation of chitosan-CMC and chitosan-pectin are listed in Table 20 in the Appendix. Drug release profiles of indomethacin microcapsules were the percentage of drug release against square root of time as Higuchi's plot. Each profile showed various conditions which affected the drug release from microcapsules. The effects of these factors such as chitosan concentration, CMC or pectin concentration, glutaraldehyde content, indomethacin content, hardening time, and calcium chloride content (only for chitosan-pectin microcapsule) were discussed in the following section. Preparations which percentage of drug release conformed to the USP specification were chitosan-CMC microcapsule formulations 2, 4 and chitosan-pectin microcapsule formulations 14, 17, 38d, 41.

a) Effect of chitosan concentration : From the drug release profiles of chitosan-CMC microcapsules shown in Figures 60-61, increasing of chitosan concentration caused lower drug release at 24 hours from microcapsules in the range of 10-19% except a slight difference in the preparations 1&6. The effect of chitosan concentration on the drug release from chitosan-pectin microcapsules shown in Figures 62-67 were the same as the drug release from chitosan-CMC microcapsules. It caused lower drug release at 24 hours in the range of 9-43%. However, the drug release of preparations 15&28 and 21&34 were faster with increasing concentration of chitosan.

b) Effect of CMC or pectin concentration : The effect of concentration of CMC or pectin on the drug release of indomethacin microcapsules are shown in Figures 60 and 68 Prep. 5,7 and 62-66, 69-70, respectively. The results were

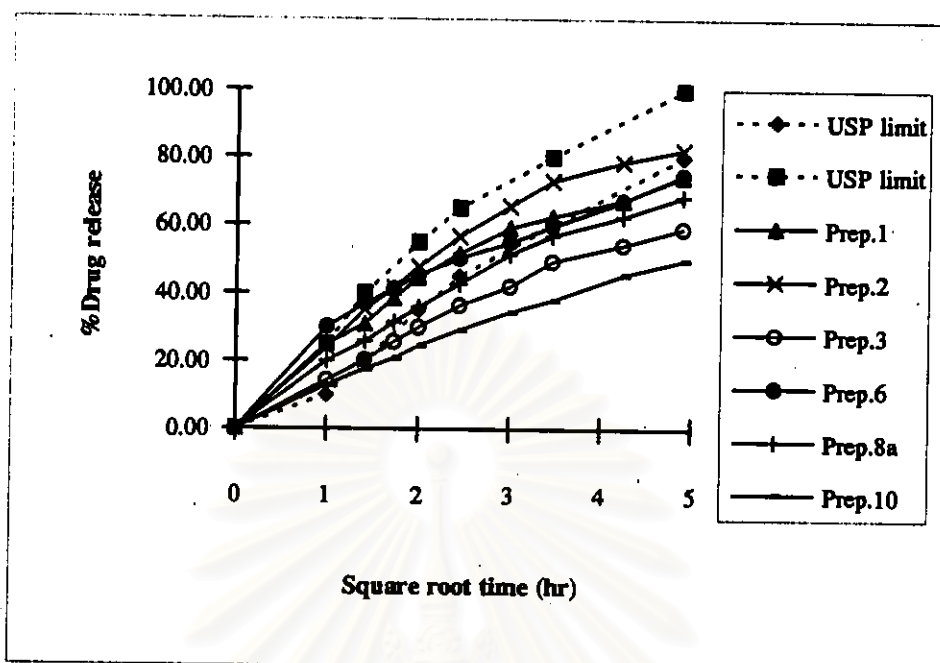


Figure 60 : Drug release profiles of chitosan-CMC indomethacin microcapsules prepared from glutaral 0.25 gm, indomethacin 1%, 2 hrs. hardening time (Prep. 1, 2, 3, 6, 8a, 10)  
 (Prep. 1, 2, 3 = chitosan 0.25% w/v, CMC 0.75%, 1%, 1.5% w/v  
 Prep. 6, 8a, 10 = chitosan 0.5% w/v, CMC 0.75%, 1%, 1.5% w/v)

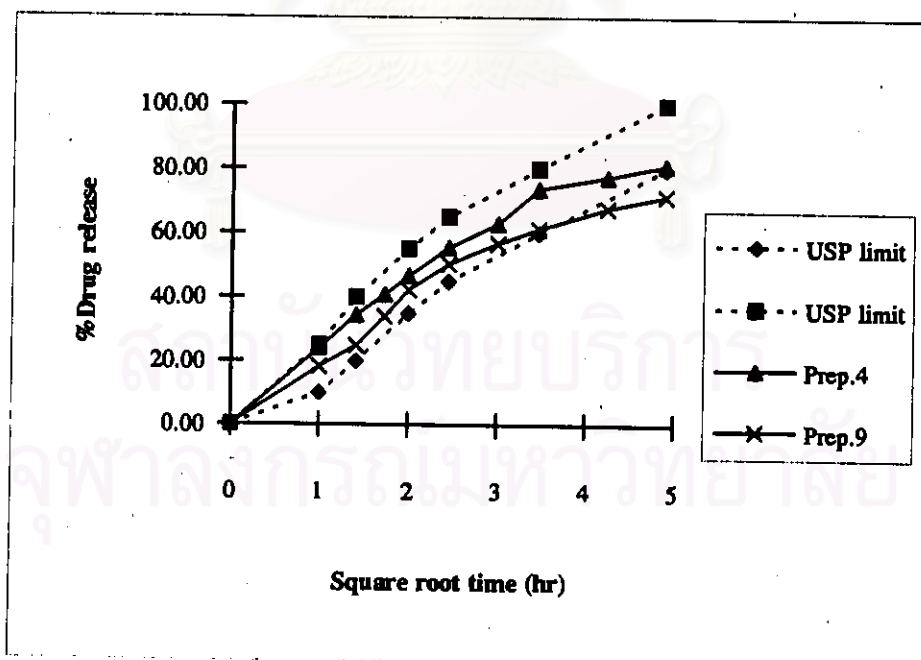


Figure 61 : Drug release profiles of chitosan-CMC indomethacin microcapsules prepared from CMC 1% w/v, glutaral 0.25 gm, indomethacin 1.5%, 2 hrs. hardening time (Prep. 4, 9)  
 (Prep. 4 = chitosan 0.35% w/v, Prep. 9 = chitosan 0.5% w/v)

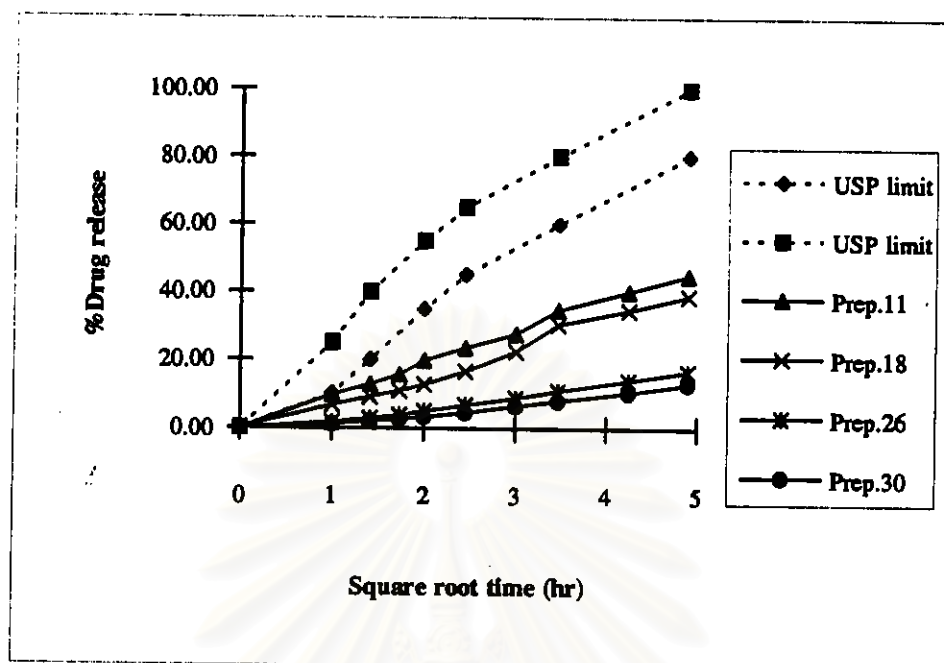


Figure 62 : Drug release profiles of chitosan-pectin indomethacin microcapsules prepared from calcium chloride 1 gm, glutaral 0.1 gm, indomethacin 1%, 2 hrs. hardening time (Prep. 11, 18, 26, 30)

(Prep. 11 and 18 = chitosan 0.25% w/v, pectin 5% and 7.5% w/v

Prep. 26 and 30 = chitosan 0.5% w/v, pectin 5% and 7.5% w/v)

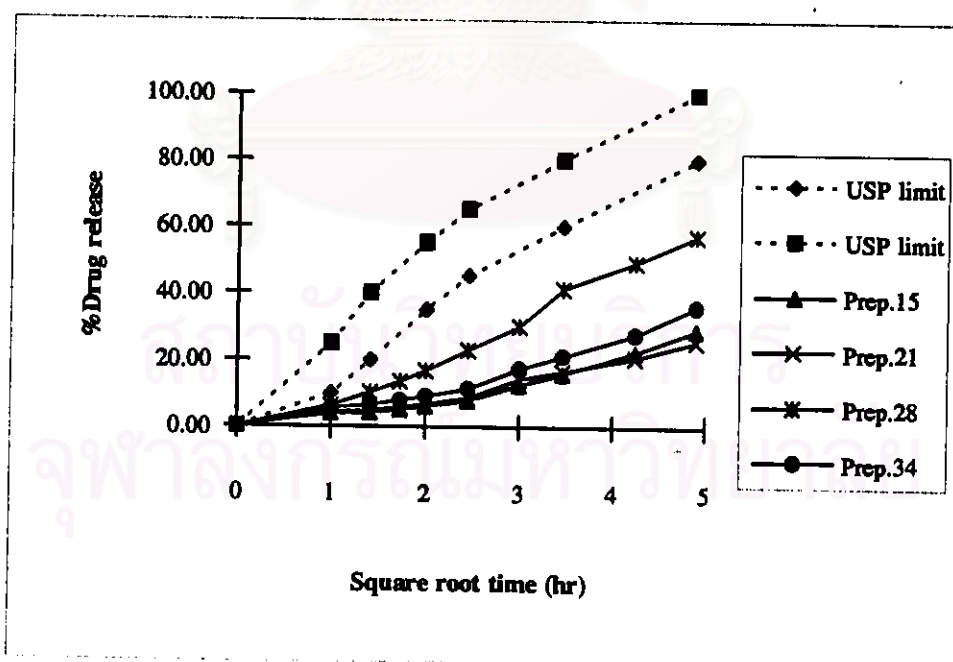


Figure 63 : Drug release profiles of chitosan-pectin indomethacin microcapsules prepared from calcium chloride 3 gm, glutaral 0.1 gm, indomethacin 1%, 2 hrs. hardening time (Prep. 15, 21, 28, 34)

(Prep. 15 and 21 = chitosan 0.25% w/v, pectin 5% and 7.5% w/v

Prep. 28 and 34 = chitosan 0.5% w/v, pectin 5% and 7.5% w/v)

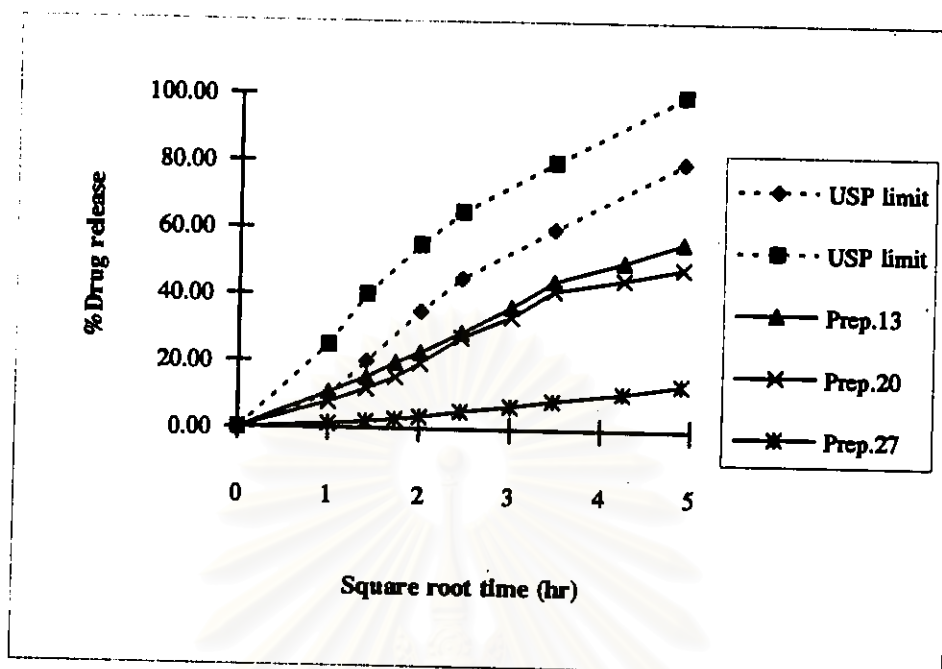


Figure 64 : Drug release profiles of chitosan-pectin indomethacin microcapsules prepared from calcium chloride 1 gm, glutaral 0.25 gm, indomethacin 1%, 2hrs. hardening time (Prep. 13, 20, 27)  
 (Prep. 13 and 20 = chitosan 0.25% w/v, pectin 5% and 7.5% w/v  
 Prep. 27 = chitosan 0.5% w/v, pectin 5% w/v)

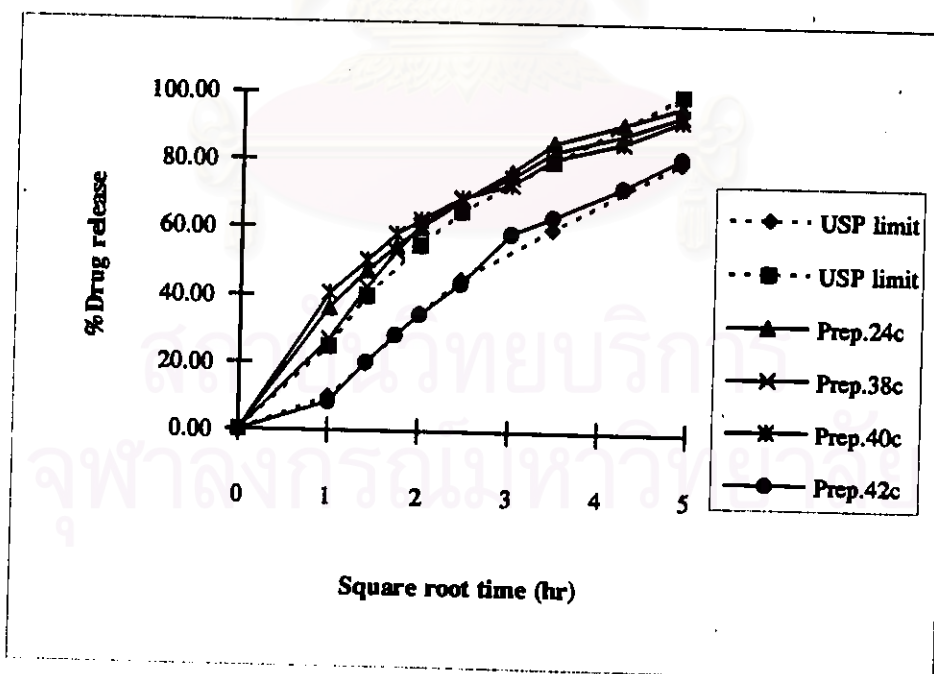
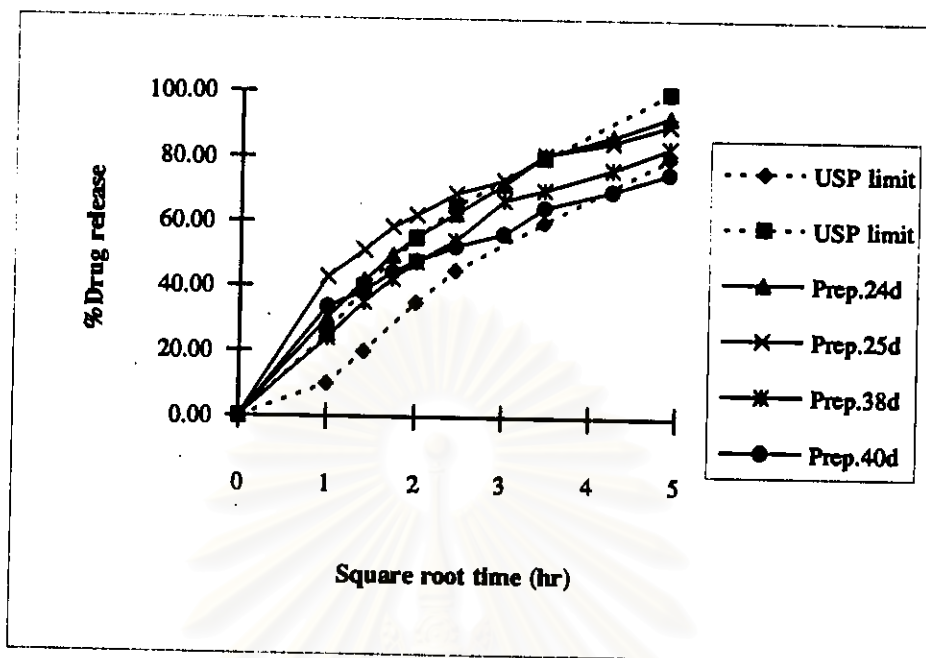
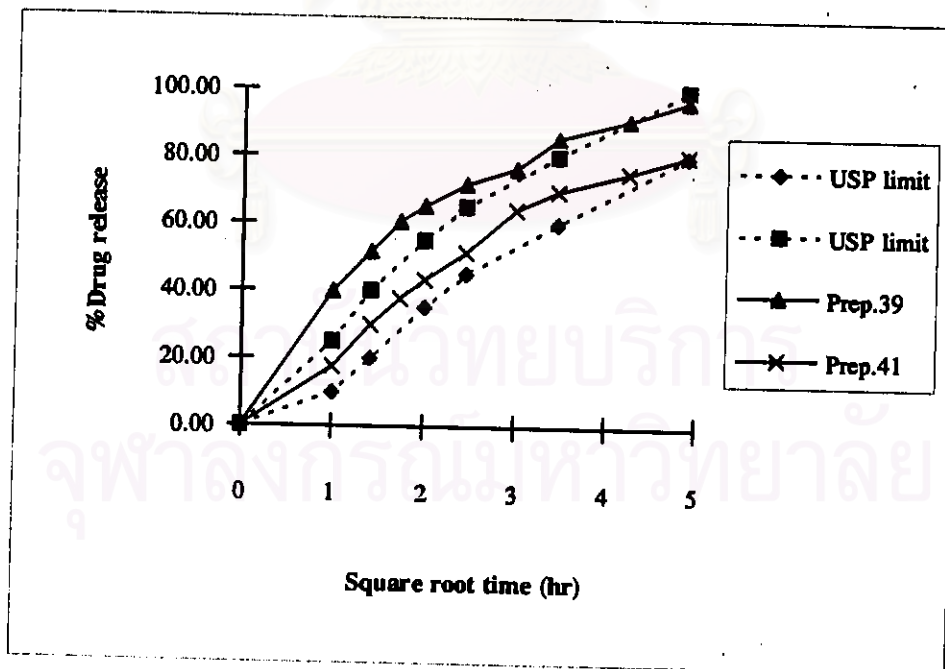


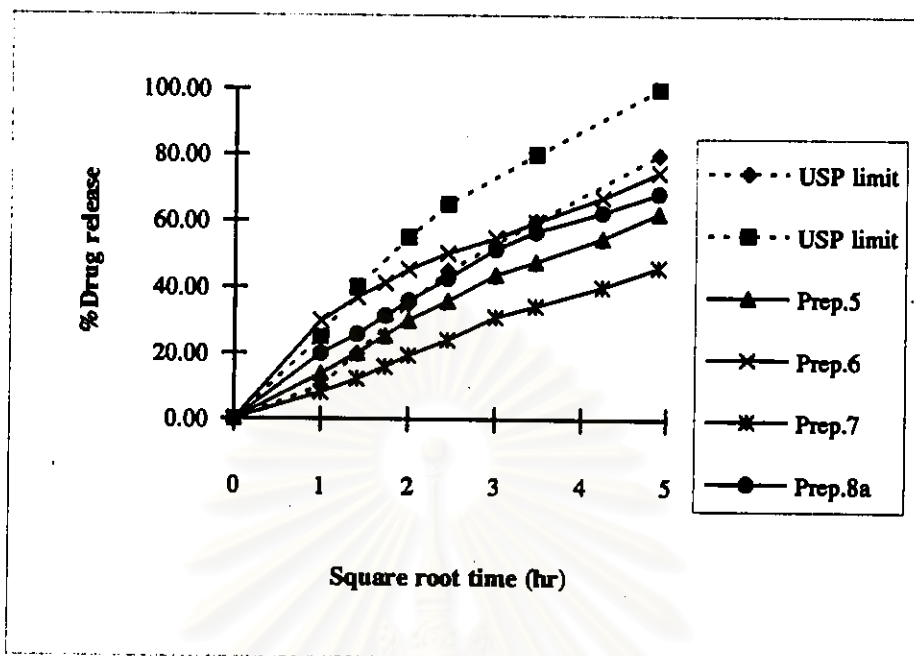
Figure 65 : Drug release profiles of chitosan-pectin indomethacin microcapsules prepared from calcium chloride 3 gm, indomethacin 3%, 6hrs. hardening time (Prep. 24c, 38c, 40c, 42c)  
 (Prep. 24c = chitosan 0.25% w/v, pectin 7.5% w/v, glutaral 0.25 gm  
 Prep. 38c and 40c = chitosan 0.5% w/v, pectin 7.5% and 10% w/v, glutaral 0.25 gm  
 Prep. 42c = chitosan 0.75% w/v, pectin 10% w/v, glutaral 0.1 gm)



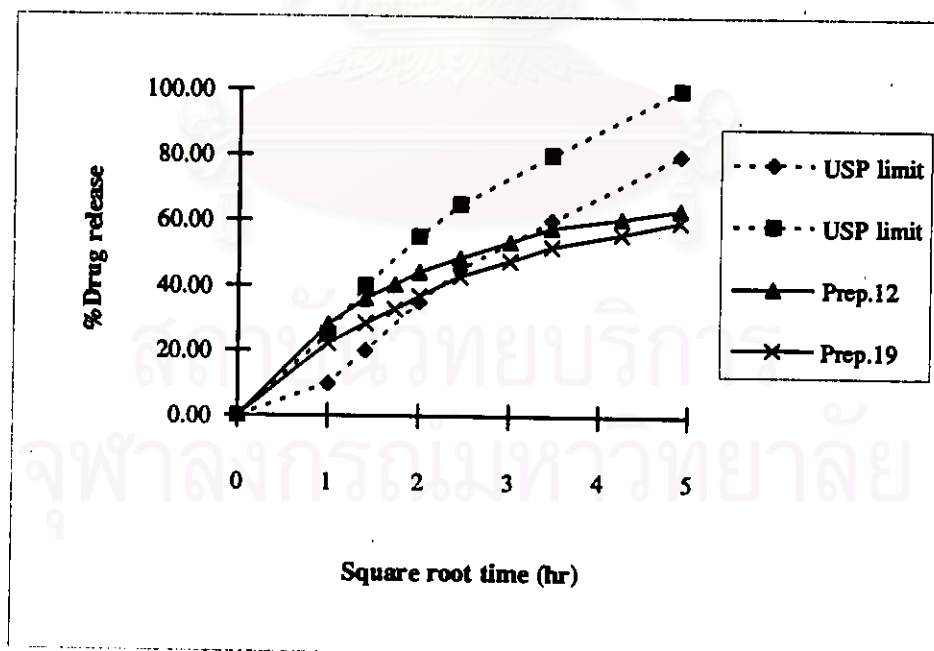
**Figure 66 :** Drug release profiles of chitosan-pectin indomethacin microcapsules prepared from calcium chloride 3 gm, glutaral 0.25 gm, indomethacin 3%, 18 hrs. hardening time (Prep. 24d, 25d, 38d, 40d)  
 (Prep. 24d and 25d = chitosan 0.25% w/v, pectin 7.5% and 10% w/v)  
 Prep. 38d and 40d = chitosan 0.5% w/v, pectin 7.5% and 10 % w/v)



**Figure 67 :** Drug release profiles of chitosan-pectin indomethacin microcapsules prepared from pectin 10% w/v, calcium chloride 3 gm, no glutaral, indomethacin 3% (Prep. 39, 41)  
 (Prep. 39 = chitosan 0.5% w/v, Prep. 41 = chitosan 0.75% w/v)

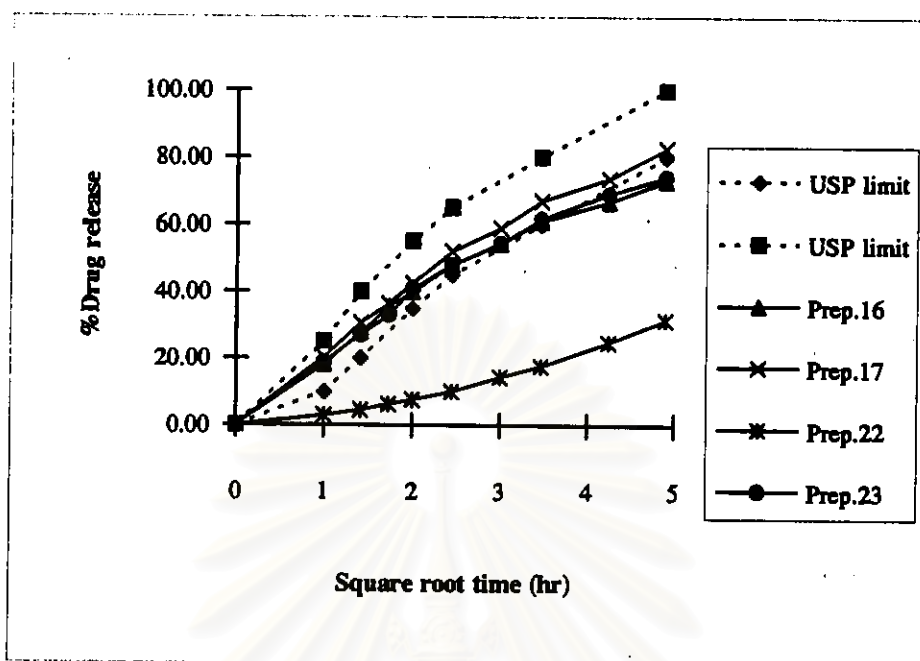


**Figure 68 :** Drug release profiles of chitosan-CMC indomethacin microcapsules prepared from chitosan 0.5%w/v, indomethacin 1%, 2 hrs. hardening time (Prep. 5, 6, 7, 8a) (Prep. 5 and 6 = CMC 0.75%w/v, glutaral 0.1 and 0.25 gm) (Prep. 7 and 8a = CMC 1%w/v, glutaral 0.1 and 0.25 gm)



**Figure 69 :** Drug release profiles of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.25%w/v, calcium chloride 1 gm, glutaral 0.1 gm, indomethacin 2%, 2 hrs. hardening time (Prep. 12, 19) (Prep. 12 = pectin 5%w/v, Prep. 19 = pectin 7.5%w/v)





**Figure 70 : Drug release profiles of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.25% w/v, calcium chloride 3 gm, glutaral 0.25 gm, 2 hrs. hardening time (Prep. 16, 17, 22, 23)**

(Prep. 16 and 17 = pectin 5% w/v, indomethacin 1% and 2%)

Prep. 22 and 23 = pectin 7.5% w/v, indomethacin 1% and 2%)

notable that increasing the concentration of CMC or pectin, the percentage of drug release decreased in the range of 4-41. But in the Figure 60, preparation 2 showed higher drug release than preparation 1 about 7% and preparation 3 about 15%, respectively.

c) Effect of glutaraldehyde content : Figures 68 and 71-74 illustrate the effect of glutaraldehyde content on drug release of chitosan-CMC and chitosan-pectin indomethacin microcapsules, respectively. The results showed that the higher glutaraldehyde content resulted in the faster release of indomethacin from both chitosan-CMC and chitosan-pectin microcapsules. Drug release of preparations which had more glutaraldehyde content of 0.25 gm increased in the range of 6-43%.

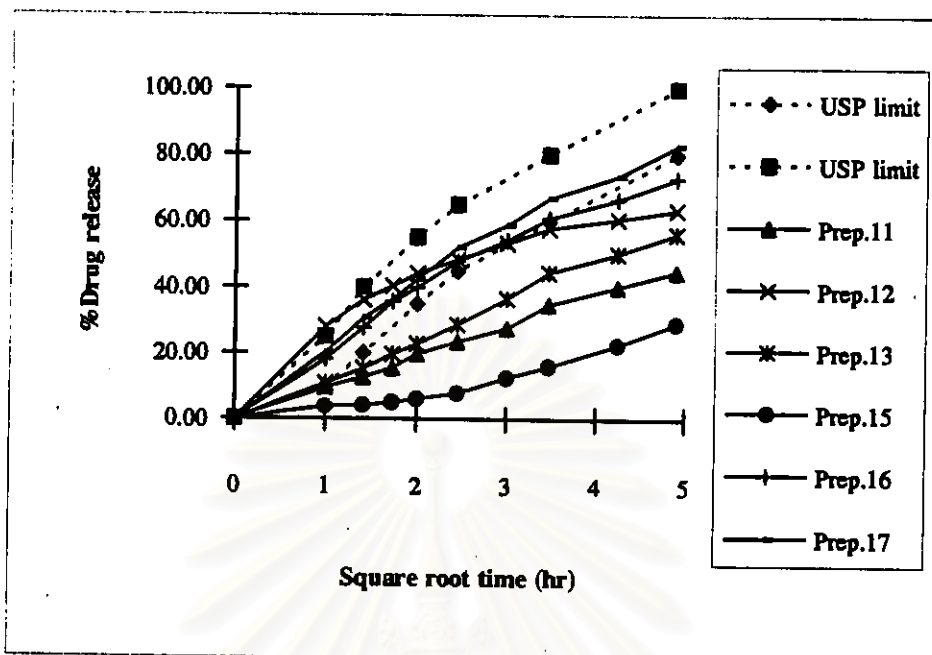


Figure 71 : Drug release profiles of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.25 %w/v, pectin 5%w/v, 2 hrs.hardening time (Prep.11,12,13,15,16,17) (Prep.11,12,13=calcium chloride 1 gm,glutaryl 0.1,0.1,0.25 gm,indomethacin 1,2,1%) Prep.15,16,17=calcium chloride 3 gm,glutaryl 0.1,0.25,0.25 gm,indomethacin 1,1,2%)

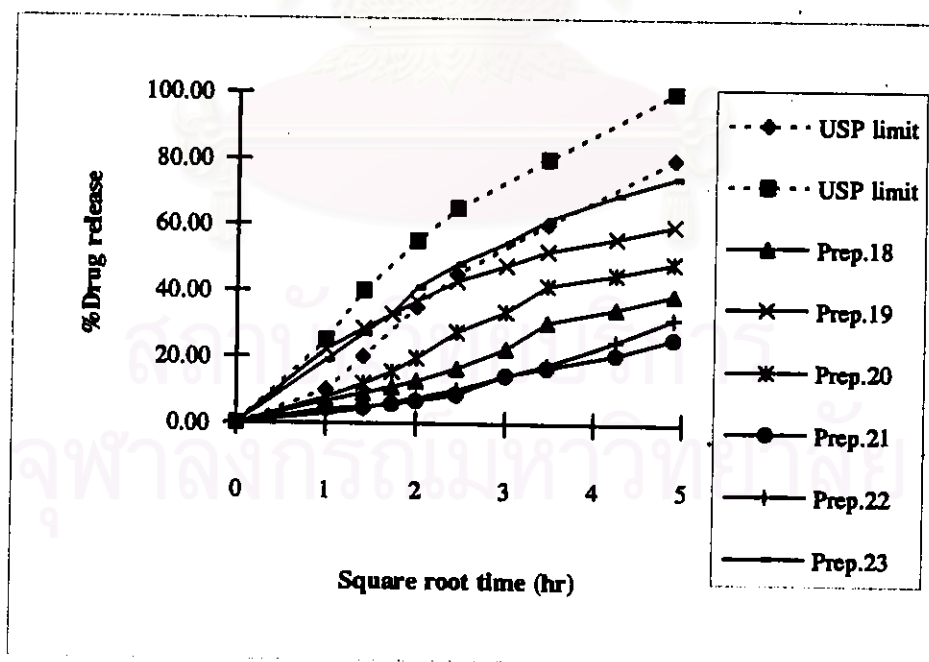
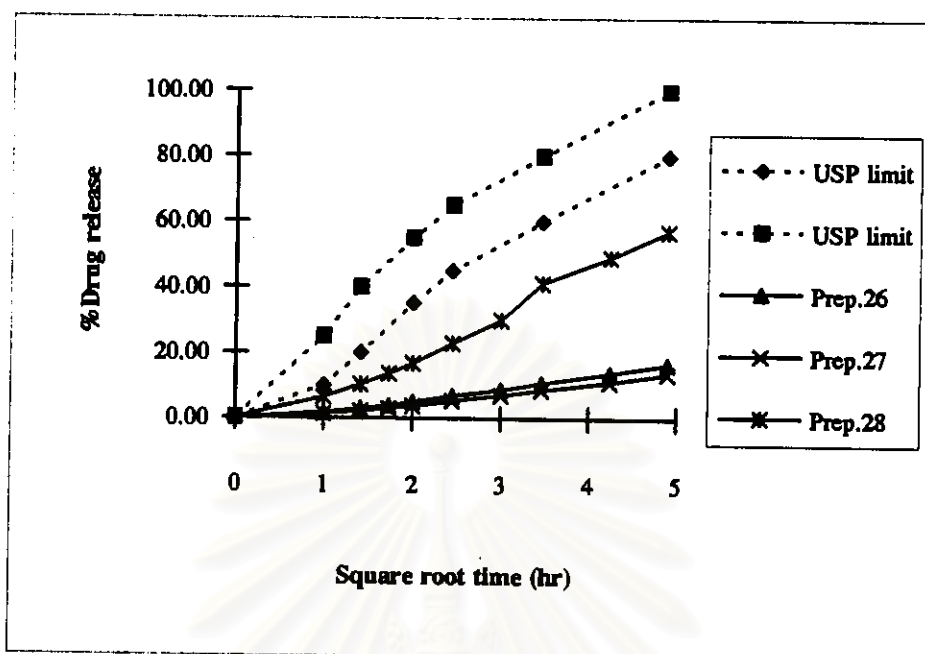
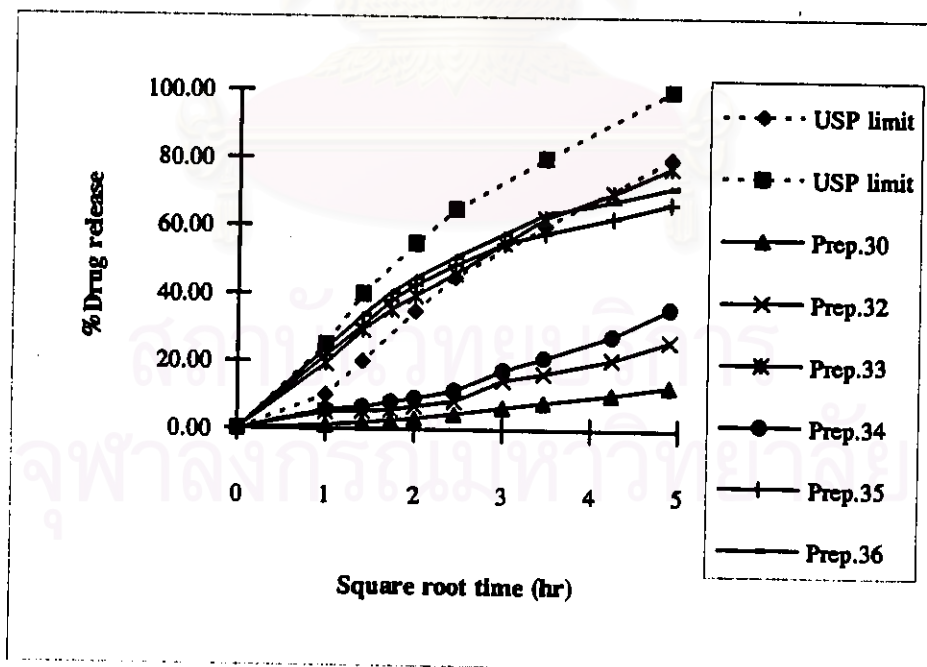


Figure 72 : Drug release profiles of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.25 %w/v, pectin 7.5%w/v, 2hrs.hardening time (Prep.18,19,20,21,22,23) (Prep.18,19,20=calcium chloride 1 gm,glutaryl 0.1,0.1,0.25 gm,indomethacin 1,2,1%) Prep.21,22,23=calcium chloride 1 gm,glutaryl 0.1,0.25,0.25 gm,indomethacin 1,1,2%)



**Figure 73 :** Drug release profiles of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.5% w/v, pectin 5% w/v, indomethacin 1%, 2 hrs. hardening time (Prep. 26, 27, 28)  
 (Prep. 26 and 27 = calcium chloride 1 gm, glutaral 0.1 and 0.25 gm  
 Prep. 28 = calcium chloride 3 gm, glutaral 0.1 gm)



**Figure 74 :** Drug release profiles of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.5% w/v, pectin 7.5% w/v, 2 hrs. hardening time (Prep. 30, 32, 33, 34, 35, 36)  
 (Prep. 30 = calcium chloride 1 gm, glutaral 0.1 gm, indomethacin 1%  
 Prep. 32 and 33 = calcium chloride 3 gm, no glutaral, indomethacin 1% and 5%  
 Prep. 34, 35, 36 = calcium chloride 3 gm, glutaral 0.1 gm, indomethacin 1, 2, 3%)

d) Effect of indomethacin content : When the content of drug increased in the preparation, the percentage of drug release also increased. Higher drug release in the range of 3-51% depended on higher indomethacin in the formulation. Their drug release profiles are shown in Figures 71-72 and 74-75.

e) Effect of hardening time : For chitosan-CMC indomethacin microcapsules, when microcapsules were hardened at 2 and 3 hours, there was no difference in the drug release profile, as shown in Figure 75. But in chitosan-pectin indomethacin microcapsules, the drug release of preparations with longer hardening time was lower in the range of 4-17% as shown in Figure 76 except Prep.24c&24d.

f) Effect of calcium chloride content : From Figures 71-74, increasing in calcium chloride content of preparations 11&15, 18&21, and 20&22 decreased drug release in the range of 13-17%. While preparations 13&16, 26&28, 30&34 increased drug release in the range of 16-41%.

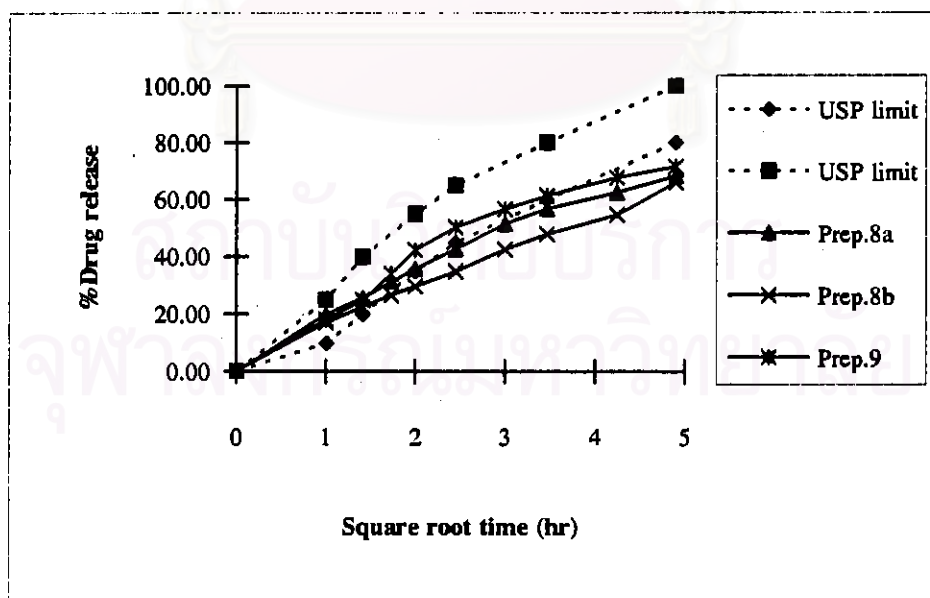


Figure 75 : Drug release profiles of chitosan-CMC indomethacin microcapsules prepared from chitosan 0.5% w/v, CMC 1% w/v, glutaral 0.25 gm (Prep.8a,8b,9) (Prep. 8a and 8b = indomethacin 1%, 2 and 3 hrs. hardening time Prep. 9 = indomethacin 1.5%, 2 hrs. hardening time)

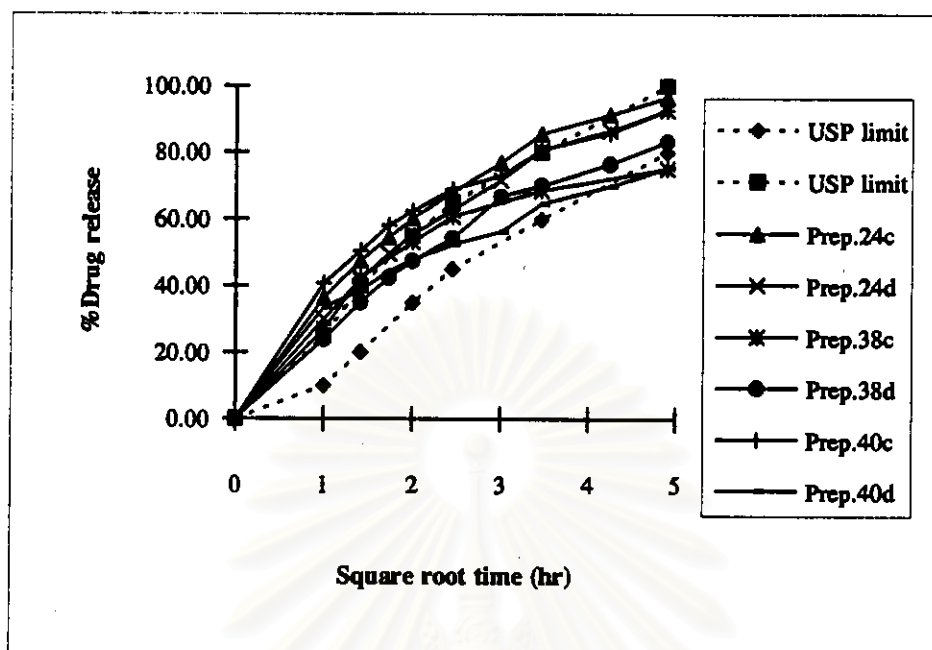


Figure 76 : Drug release profiles of chitosan-pectin Indomethacin microcapsules prepared from calcium chloride 3 gm,glutaral 0.25 gm,indomethacin 3% (Prep.24c,d,38c,d,40c,d) (Prep.24c and 24d=chitosan 0.25%w/v,pectin 7.5%w/v,6 and 18 hrs.hardening time Prep.38c and 38d=chitosan 0.5%w/v,pectin 7.5%w/v,6 and 18 hrs.hardening time Prep.40c and 40d=chitosan 0.5%w/v,pectin 10%w/v,6 and 18 hrs.hardening time)

## 2.5 Determination of infrared spectra

The infrared spectra of chitosan, CMC, pectin,  $\text{CaCl}_2$  and indomethacin are shown in Figure 77. Bands in the region from  $3700$  to  $3100 \text{ cm}^{-1}$  were various OH stretching vibrations. Chitosan showed absorption peak of  $\text{NH}_2$  stretching at about  $1590 \text{ cm}^{-1}$ . Absorption peaks at  $1690$  and  $1710 \text{ cm}^{-1}$  of indomethacin were assigned to stretching vibration of carbonyl groups COO and NCO, respectively. Figure 78 shows infrared spectra of chitosan-CMC, chitosan-CMC with glutaraldehyde, chitosan-CMC with indomethacin, and chitosan-CMC indomethacin microcapsules. There were absorption peaks of CONH stretching at  $1710$ - $1580 \text{ cm}^{-1}$  when it was determined from chitosan-CMC complex coacervate and chitosan-CMC microcapsule. When there was notable absorption band of chitosan-CMC indomethacin complex coacervate, it showed obviously absorption peaks of

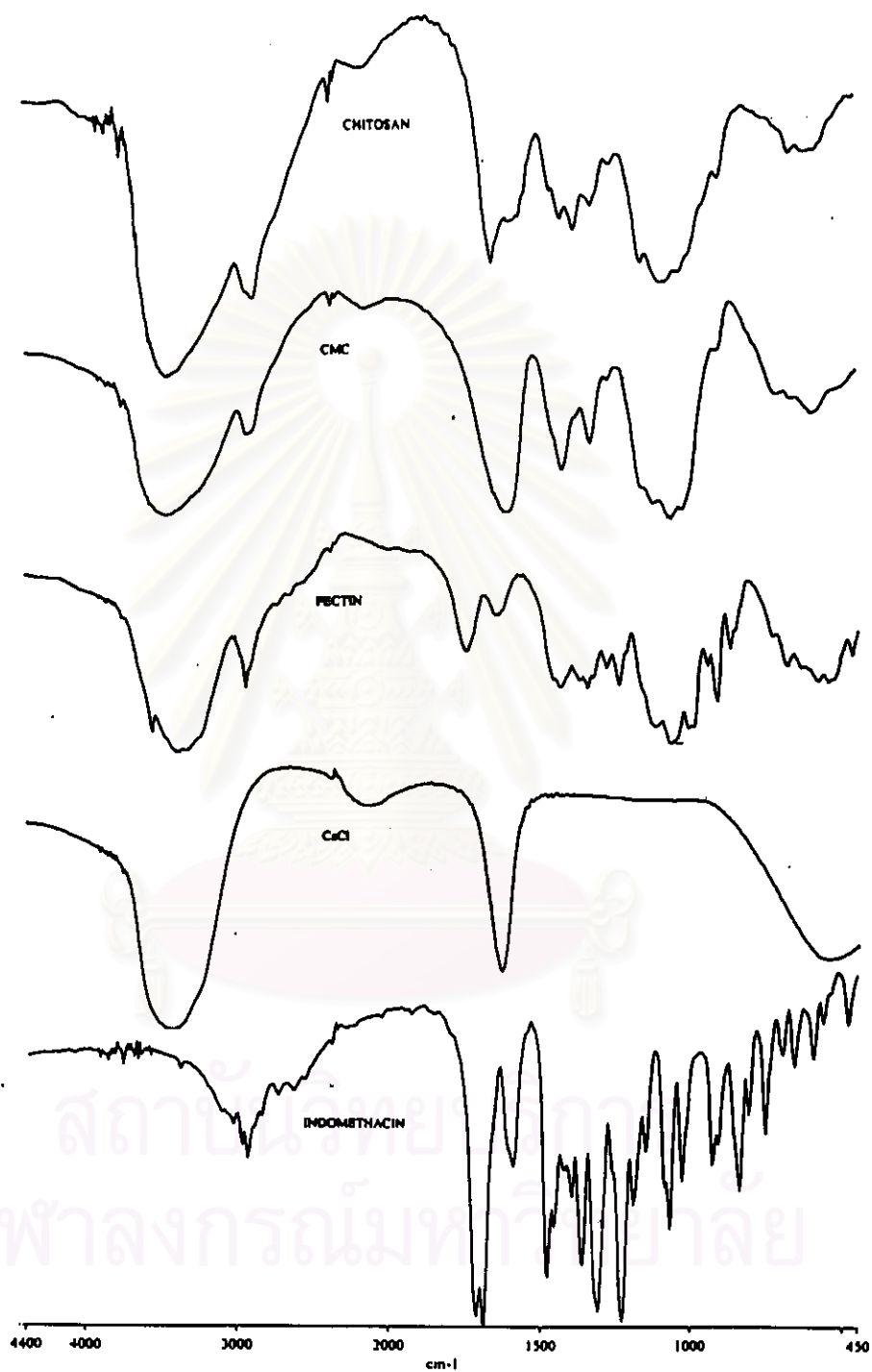
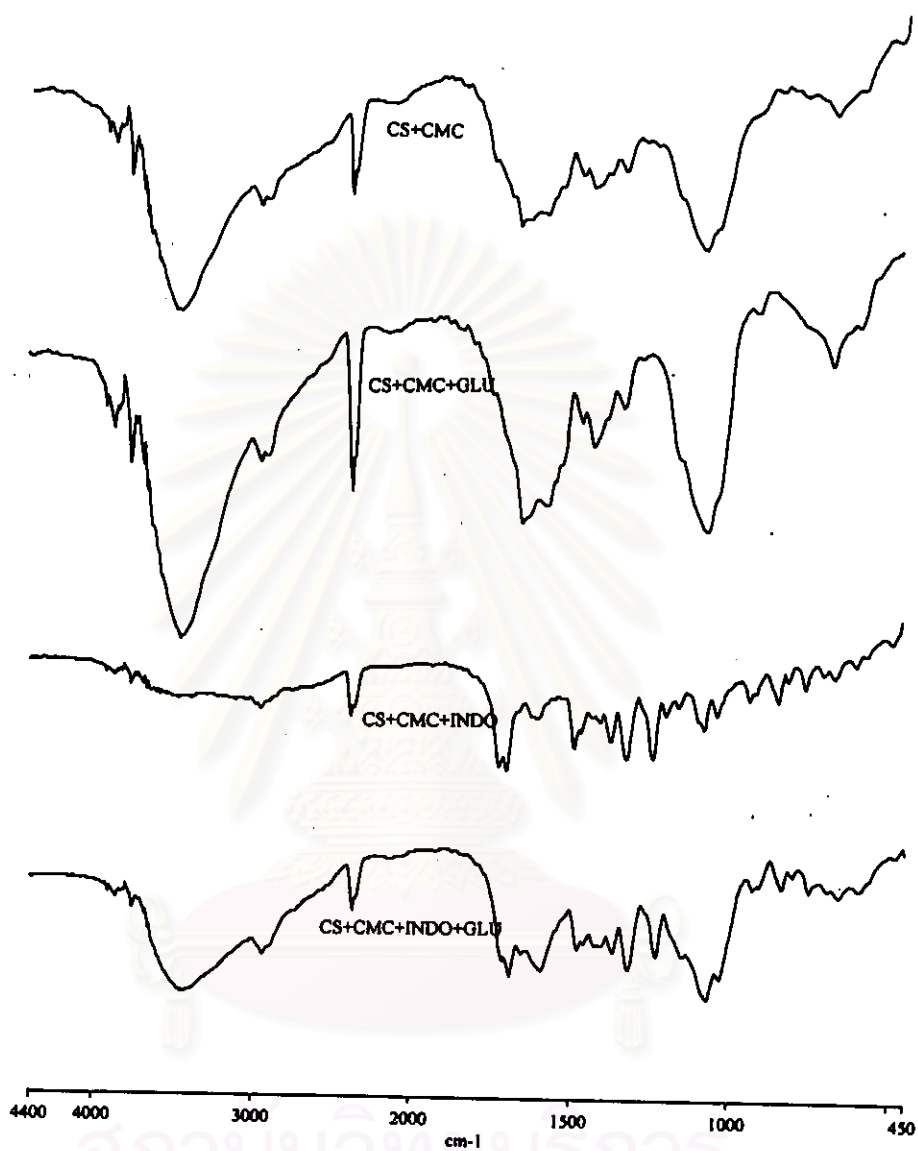


Figure 77 : Infrared spectra of chitosan, CMC, pectin, CaCl<sub>2</sub>, and indomethacin



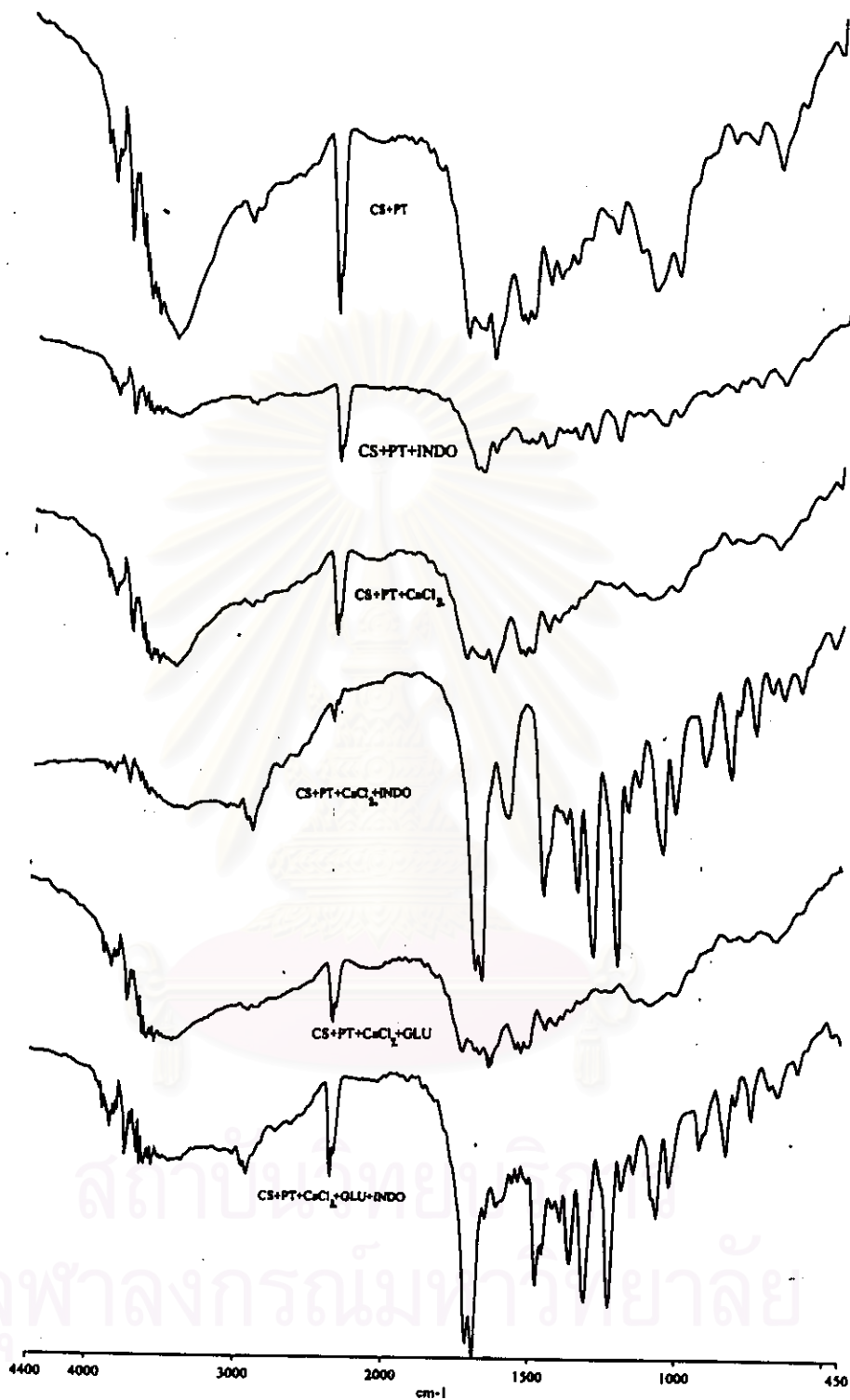
**Figure 78 : Infrared spectra of chitosan-CMC complex coacervate or microcapsule with or without indomethacin**

indomethacin interfering the peaks of interaction of chitosan-CMC complex coacervate. In chitosan-CMC indomethacin microcapsules, it showed absorption peaks of CONH stretching at  $1710-1580\text{ cm}^{-1}$ . The infrared spectra of chitosan-pectin complex coacervate or microcapsules with or without indomethacin are displayed in Figure 79. They showed absorption peaks of CONH stretching at  $1710-1580\text{ cm}^{-1}$ . The peaks of indomethacin could be seen in the preparation that added indomethacin into pectin solution hence these peaks interfered the absorption peak of CONH stretching. According to IR spectra of preparations chitosan-pectin with or without calcium chloride, they could be found similar absorption band. The spectra of preparations with or without glutaraldehyde of chitosan-CMC and chitosan-pectin without indomethacin showed absorption peaks of C=N stretching at  $1660-1590\text{ cm}^{-1}$ .

## 2.6 Determination of differential scanning calorimetric thermogram

Thermograms of chitosan, CMC, pectin, chitosan-CMC, chitosan-pectin and chitosan-pectin with calcium chloride are shown in Figure 80. Endothermic peak of chitosan was at about  $155^{\circ}\text{c}$ . Pectin displayed endothermic peak at  $172.8^{\circ}\text{c}$ . For chitosan-CMC complex coacervate with chitosan  $0.25\%w/v$  and CMC  $1\%w/v$ , endothermic peak was shifted to lower temperature at about  $75^{\circ}\text{c}$ . The thermograms of chitosan-pectin complex coacervate with chitosan  $0.25\%w/v$ , pectin  $5\%w/v$ , and with or without calcium chloride  $3\text{ gm /gm}$  chitosan were seen to have shifting endothermic peak below at  $117.2^{\circ}\text{c}$  and broad peak at about  $115^{\circ}\text{c}$ , respectively, which two thermograms almost had the same peak. From the thermograms of complex coacervate, they could be observed that the endothermic peaks of chitosan, CMC, or pectin disappeared.





**Figure 79 : Infrared spectra of chitosan-pectin complex coacervate or microcapsule with or without indomethacin**

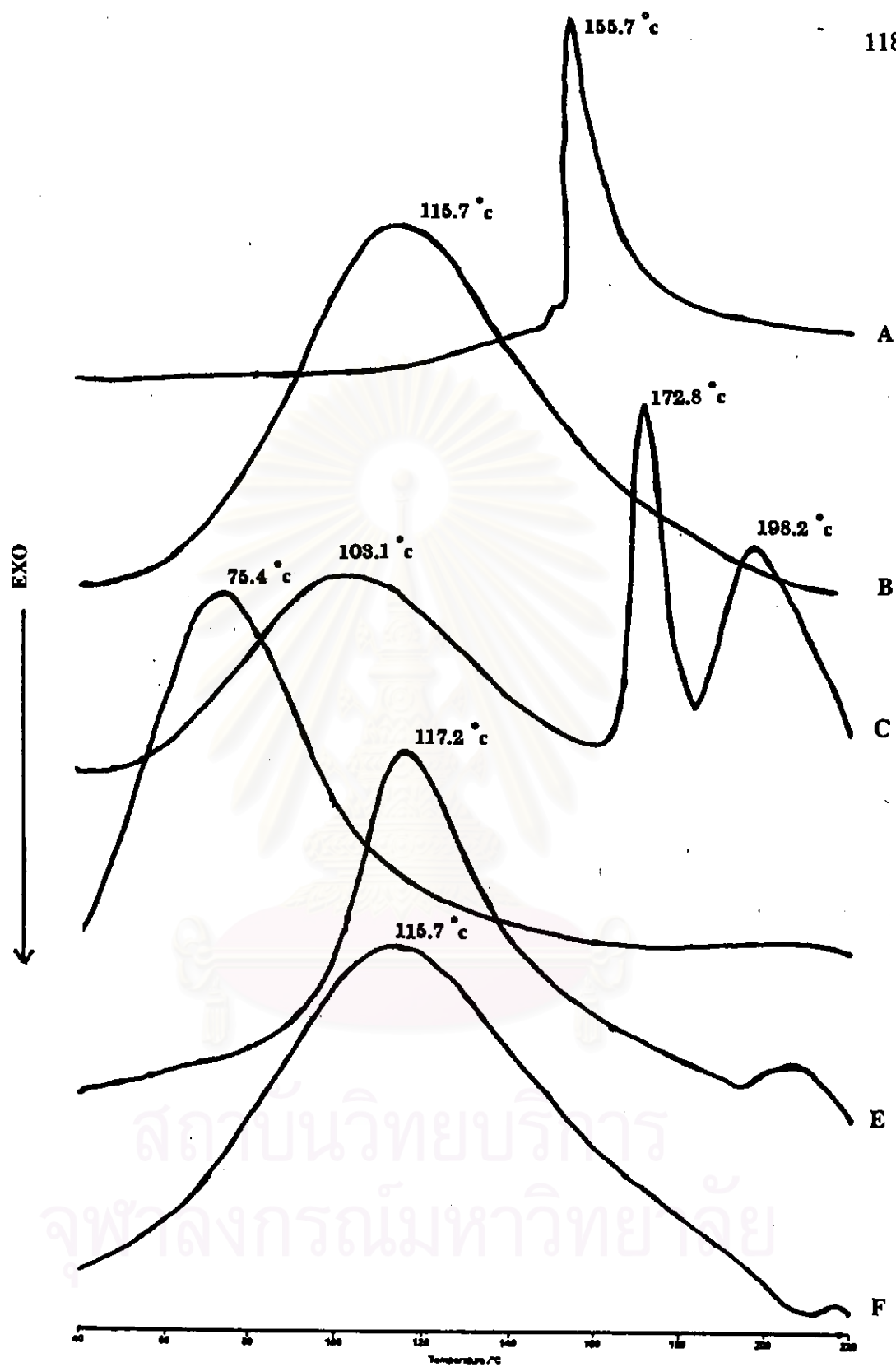


Figure 80 : DSC thermograms of chitosan (A), CMC (B), pectin (C), chitosan-CMC (D), chitosan-pectin (E), and chitosan-pectin with  $\text{CaCl}_2$  (F), complex coacervate.

### 8. Reproducibility study

Preparations 2 and 17 which conformed to the USP drug release specification were chosen for the study of reproducibility. The two preparations were representative of chitosan-CMC and chitosan-pectin indomethacin microcapsules, respectively. Determination of physical properties and drug release patterns were performed in three batches of each preparation.

Morphology of indomethacin microcapsules was determined by scanning electron microscope. The scanning electron photomicrographs of three batches of preparations 2 and 17 are shown in Figures 81 and 82 respectively. The photomicrographs of all batches of preparation 2 prepared from chitosan-CMC show smooth surface with a little wrinkled on the membrane. The three photomicrographs of chitosan-pectin indomethacin microcapsules illustrated in Figure 82 show rather rough surface and very wavy form.

The geometric mean diameters ( $D_{50}$ ) of indomethacin microcapsules prepared from preparations 2 and 17 is shown in Table 15. In preparation 2,  $D_{50}$  values varied in the range of 52.48-61.66  $\mu\text{m}$ . While  $D_{50}$  values of preparation 17 varied in the range of 208.93-239.88  $\mu\text{m}$ . Figures 83 and 84 show the size distributions of chitosan-CMC and chitosan-pectin indomethacin microcapsules, respectively. Each batch of preparation 2 displayed similarly narrow skewed size distributions and varied in the range of 40-249  $\mu\text{m}$  shown in Figure 83. The size distributions of preparation 17 shown in Figure 84 exhibited wider size distributions in three different batches and varied in the range of 82-459  $\mu\text{m}$ .

Indomethacin microcapsules from preparations 2 and 17 were evaluated for

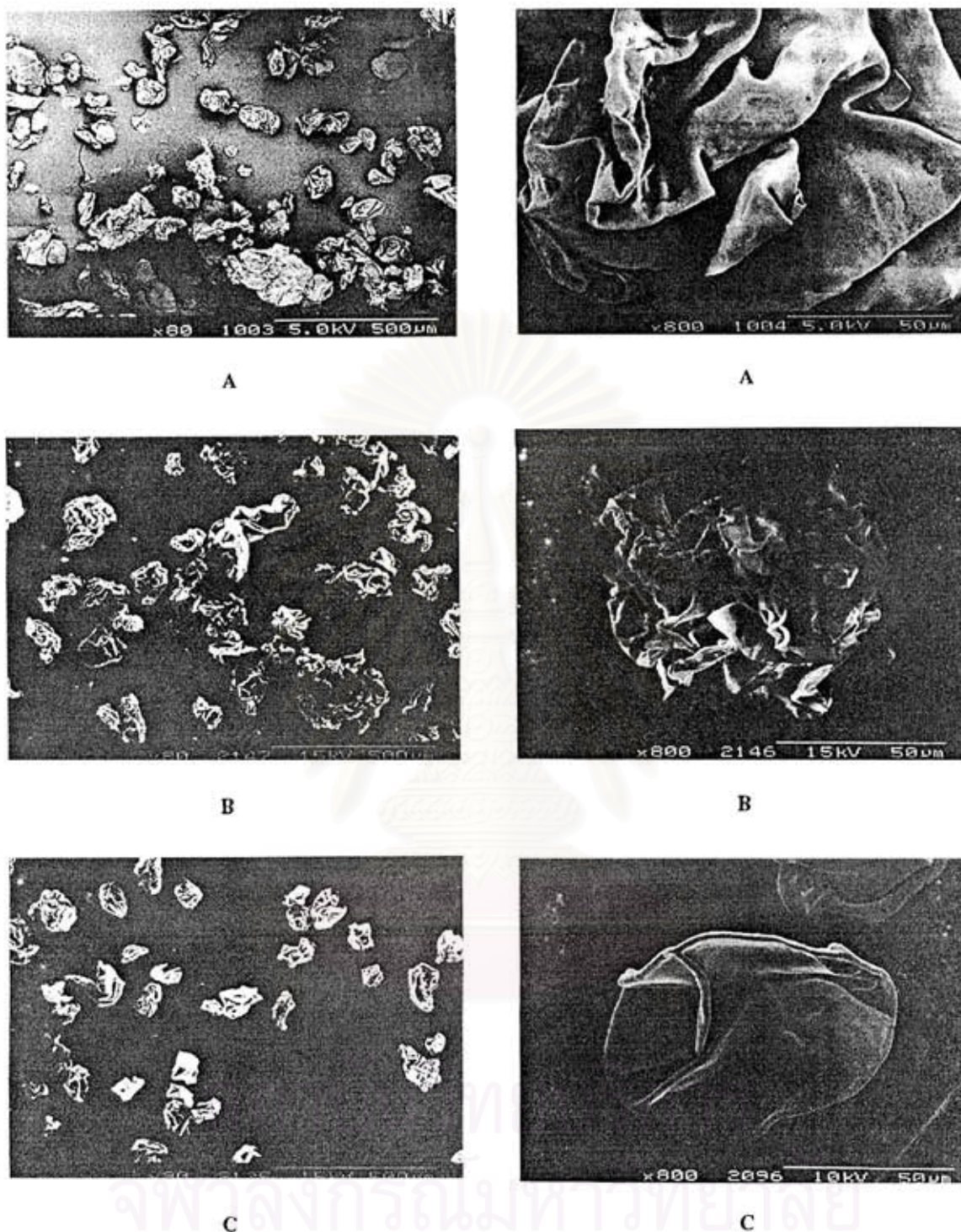
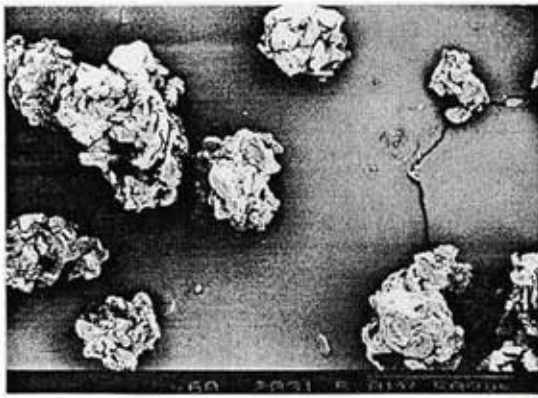
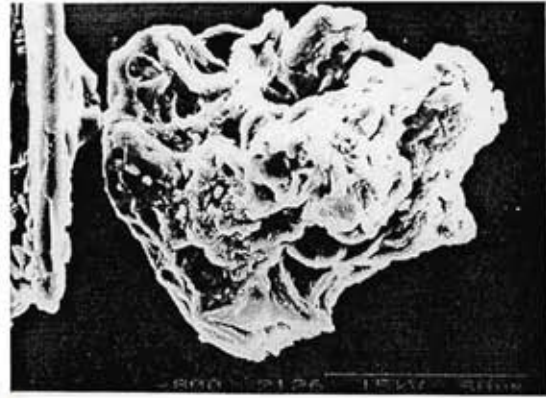


Figure 81 : Scanning electron photomicrographs of chitosan-CMC indomethacin microcapsules prepared from chitosan 0.25%w/v, CMC 1%w/v, glutaral 0.25 gm, 2 hrs. hardening time (prep. 2), x80 and x800 magnification (A = batch I, B = batch II, C = batch III)



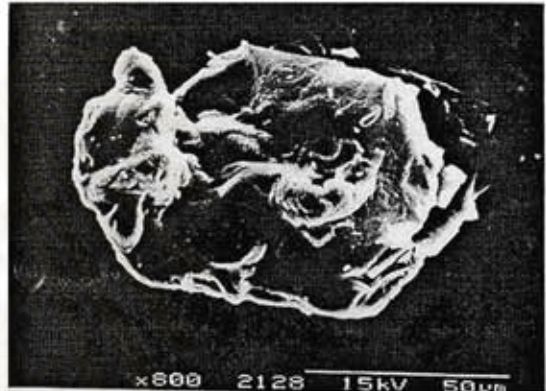
A



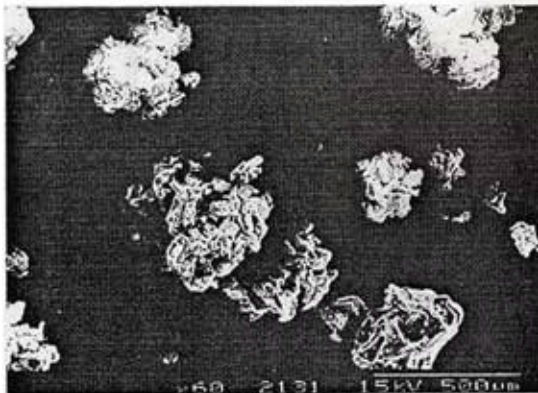
A



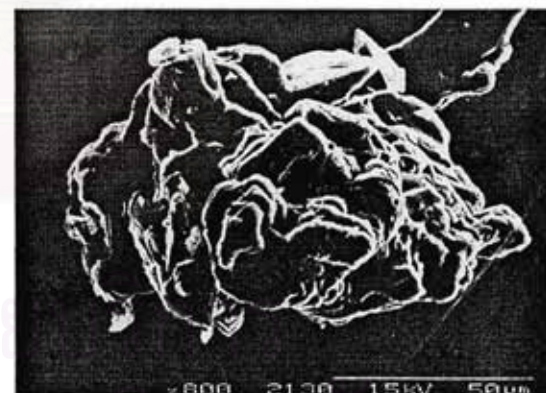
B



B



C



C

Figure 82 : Scanning electron photomicrographs of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.25%w/v, pectin 5.0%w/v, calcium chloride 3 gm, glutaral 0.25 gm, indomethacin 2%, 2 hrs. hardening time (prep. 17), x60 and x800 magnification (A = batch I, B = batch II, C =batch III)

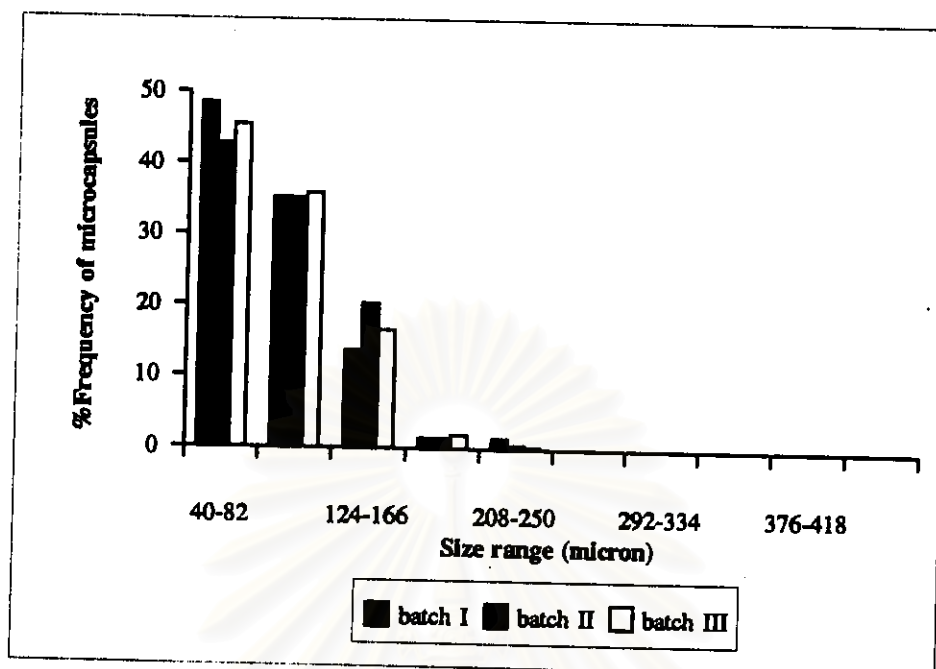


Figure 83 : Size distributions of chitosan-CMC indomethacin microcapsules prepared from chitosan 0.25% w/v, CMC 1% w/v, glutaral 0.25 gm, 2 hrs. hardening time in various batches of Preparation 2

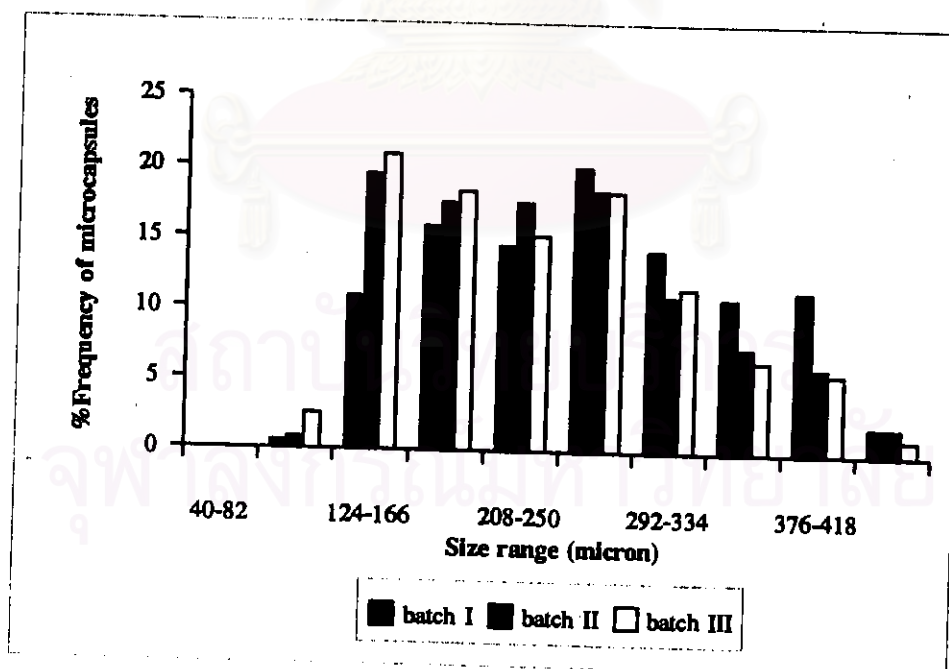


Figure 84 : Size distributions of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.25% w/v, pectin 5% w/v, calcium chloride 3 gm, glutaral 0.25 gm, indomethacin 2%, 2 hrs. hardening time in various batches of Preparation 17

**Table 15 : Geometric mean diameters ( $D_{50}$ ) of indomethacin microcapsules prepared from various batches of preparations 2 and 17**

Preparation	$D_{50}$ (micron)
2 batch I	52.48
2 batch II	61.66
2 batch III	56.23
17 batch I	239.88
17 batch II	234.42
17 batch III	208.93

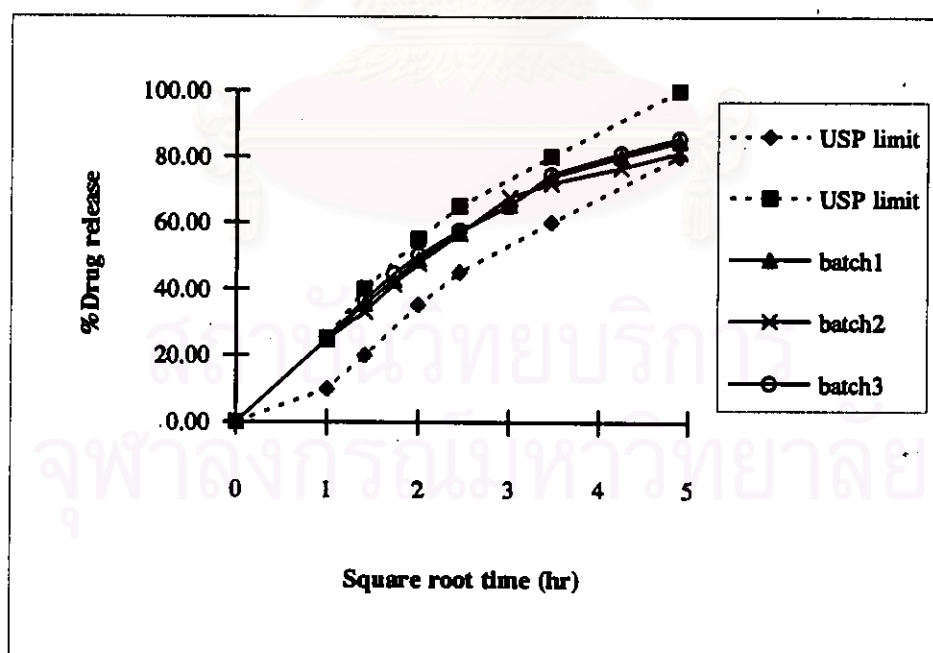
the percentage of drug entrapment and drug recovery and the results are presented in Table 16. It could be found that three batches of each preparation showed the narrow range of the percentage of drug entrapment, drug recovery and yield. The preparation 2 had drug entrapment and drug recovery in the range of 35.24 -35.91% and 71.36-74.40%, respectively. In the fourth batch of preparation 2, the process of filtering was improved by using paper filter no. 1 instead of cotton filter to decrease loss of the yield. So, drug recovery and yield of this batch were increased to 84.51% and 56.73% respectively. The drug entrapment, drug recovery and yield of chitosan-pectin indomethacin microcapsules in preparation 17 ranged from 26.51-28.40%, 75.13-78.30%, and 60.16-64.50%, respectively.

Drug release profiles of indomethacin microcapsules preparations 2 and 17 are shown in Figures 85 and 86. The results in three batches of both preparations showed no difference in the drug release profiles of Higuchi's plot. Both preparations prepared from chitosan-CMC and chitosan-pectin indomethacin microcapsules conformed to the USP drug release specification.

**Table 16 : Percentages of drug entrapment, drug recovery, and yield of indomethacin microcapsules prepared from various batches of preparations 2 and 17.**

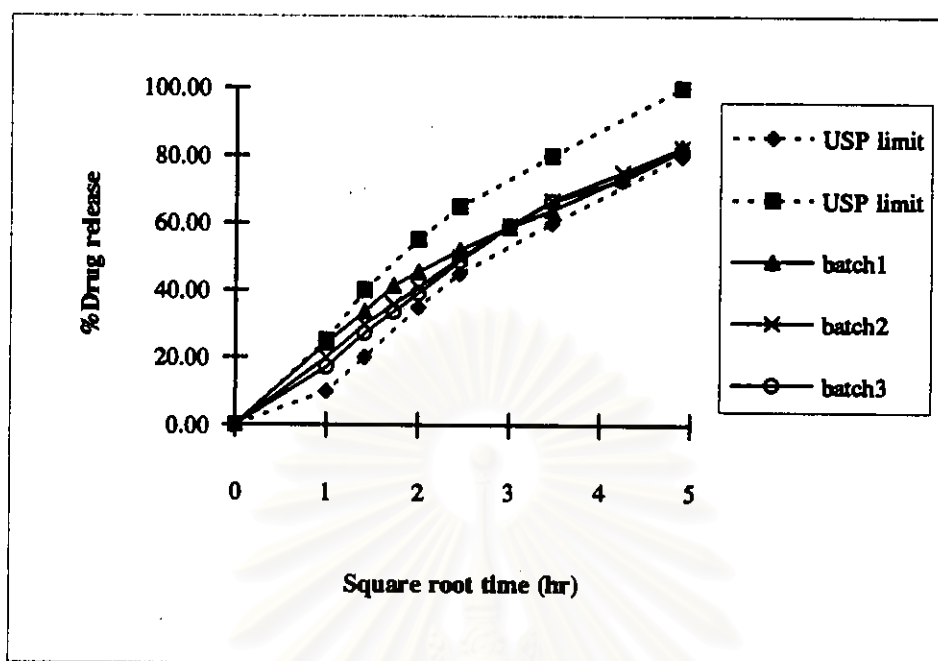
Preparation	%Drug entrapment	%Drug recovery	% Yield
2 batch I	35.85	72.15	49.29
2 batch II	35.24	71.36	49.59
2 batch III	35.91	74.40	50.73
2 batch IV*	35.58	84.51	56.73
17 batch I	26.51	77.65	64.50
17 batch II	28.40	78.30	60.72
17 batch III	27.50	75.13	60.16

\* The batch was filtered with paper filter no. 1 instead of cotton filter.



**Figure 85 : Drug release profiles of chitosan-CMC indomethacin microcapsules prepared from chitosan 0.25%w/v, CMC 1%w/v, glutaral 0.25 gm, 2 hrs. hardening time in various batches of Preparation 2**





**Figure 86 : Drug release profiles of chitosan-pectin indomethacin microcapsules prepared from chitosan 0.25%w/v, pectin 5%w/v, calcium chloride 3 gm, glutaral 0.25 gm, indomethacin 2%, 2 hrs. hardening time in various batches of Preparation 17**

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