Chapter 3

Results

1. Preliminary Studies

The results showed that microcapsules could be successfully prepared by this method of coacervation technique. Table 7 showed the formability of chitosan-CMC microcapsules at various processing conditions. These were the concentration and the pH of chitosan solution, concentration of CMC solution, and temperature of chitosan solution. From this Table it indicated that at all concentrations of 0.25-1.0% w/v and all pH's of 3, 4, and 5 of chitosan solution, the micro capsules could be prepared with CMC solution of 1.0 and 2.0% w/v and at temperature of 5 and 15°C. When the temperature was raised to 25°c, microcapsules was partially formed. Some of them trended to coalesce together and formed chain, which can be seen as white cluster form. It was shown that the microcapsules could not prepared at any processing conditions when 0.5 % w/v of CMC solution was used instead a white thread like mass was found

2. Pharmaceutical Microcapsules

The dried pharmaceutical microcapsules looked like granules of pale yellow colour. From the observation, microcapsules prepared from pH 3 of chitosan solution showed flowability characteristic better than those prepared from pH 4 and pH 5 of chitosan solution respectively. Microcapsules prepared from pH 5 of chitosan solution took longer drying time and were observed to be more swell than others while with pH 3 of chitosan solution the collapsing of microcapsules was more evident.

Table 7 : Formability of chitosan-CMC microcapsule at various processing condition

Chitosan solution conc. (%w/v)	CMC solution conc. (%w/v)	Processing Variables								
		5°C			15°C			25°C		
		pH 3	pH 4	pH 5	pH 3	pH 4	pH 5	pH 3	pH 4	pH 5
0.25	0.5	N	N	N	N	N	N	N	N	N
	1.0	E	E	E	E	E	E	P	P	P
	2.0	E	E	E	E	E	E	P	P	P
0.50	0.5	N	N	N	N	N	N	N	N	N
	1.0	E	E	E	E	E	E	P	P	P
	2.0	E	E	E	E	E	E	P	P	P
1.0	0.5	N	N	N	N	N	N	N	N	N
	1.0	E	E	E	E	E	E	P	P	P
	2.0	E	E	E	E	E	E	P	P	P

N = Non Encapsulation P = Partially Encapsulation E = Encapsulation

The results also showed that microencapsulation could be achieved with all the processing condition used. However some preparations fail to give collected yield in the recovery process, as shown in Table 8.

From preparations 9, 10, 12 and 20, after washing with IPA and then drying under nitrogen gas, the microcapsules adhered together to form a tacky agglomerate. The drying time for these preparations were more than 3 hours. While with preparations 23, 26 and 27, after some determined hardening time the medium formed gel. With the microcapsules trapped in the gel medium the yields could not be collected.

Table 8 : Appearance of indomethacin microcapsules which fail in recovery process

Preparation	Chitosan Soln pH	Hardening time (hr)	Glutaral content(gm)	Microcapsule appearance
9	3	1	0.25	Tacky and agglomerate
10	3	1	0.5	
12	4	1	0.25	
20	5	1	0.25	
23	5	1	1.5	Deposited in gel like medium
26	5	3	1.0	
27	5	3	1.5	

2.1 Morphology of Pharmaceutical Microcapsule

Figures 12A, 12B, and 12C showed the optical photomicrographs of pindolol microcapsules prepared at pH 3, pH 4 and pH 5 of chitosan solution respectively. The photomicrographs showed that the yielded microcapsules entrapped none or small amount of pindolol at all pH of chitosan solution.

The optical photomicrographs of indomethacin were shown in Figures 13A, 13B and 13C, the microcapsules were prepared from pH 3, pH 4 and pH 5 of chitosan solution respectively with glutaraldehyde 0.25 gm and 3 hr hardening time. It could be seen that the yielded microcapsules entrapped drug successfully and they were of multinuclear structure at all pH of chitosan solution. However microcapsules from pH 3 and pH 4 of chitosan solution entrapped more drug in each microcapsule more than those microcapsules prepared from pH 5 of chitosan solution. Additionally microcapsules from pH 5 solution showed spherical shape while microcapsules from pH 3 and pH 4 solution showed irregular shape. By observation, the wall of microcapsules from pH 5 solution was thinnest, also it could be seen that the membrane was ruptured or dissolved with time when mounted with water and observed under optical microscope.

The collapsing behaviour of microcapsules varied with the different pH of chitosan solution used for the preparation of microcapsules. With chitosan solution of pH 3 the microcapsule immediately started to collapse during the hardening period while with the pH 5 of chitosan solution it did not collapse until washed with IPA. For microcapsules prepared with pH 4 of chitosan solution some started collapsing during hardening period and some only when washed with IPA.

Figure 14 showed photomicrographs of chitosan, CMC, and indomethacin. It appeared that the particle of chitosan and CMC were irregular in shape. Indomethacin appeared to be regular crystalline, generally square shape with various sizes and glossy surface.

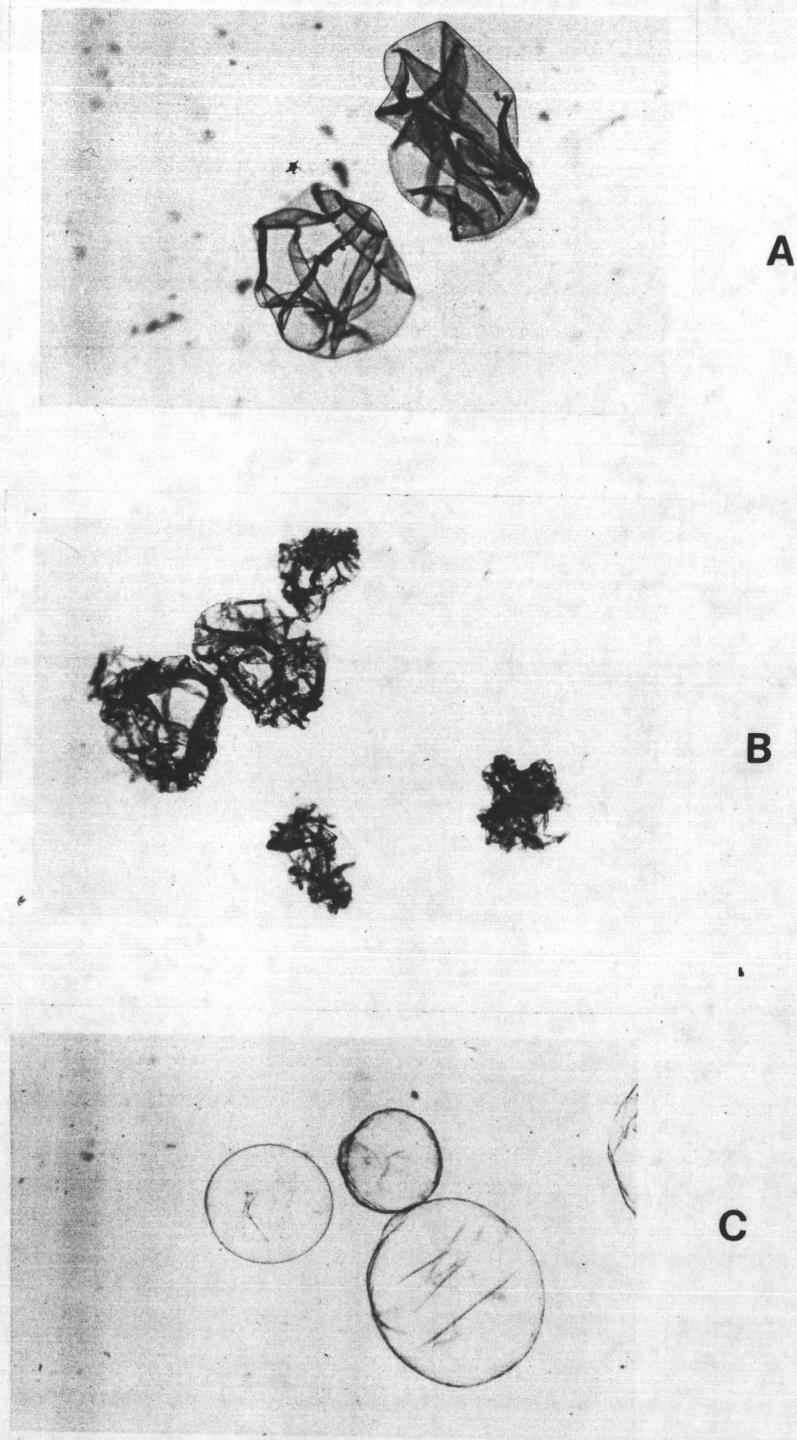


Figure 12 : Optical photomicrograph of pindolol microcapsule prepared from glutaral 0.25 gm, 3 hr hardening time, x200 magnification

- **A : chitosan solution pH3**
- **B : chitosan solution pH4**
- **C : chitosan solution pH5**

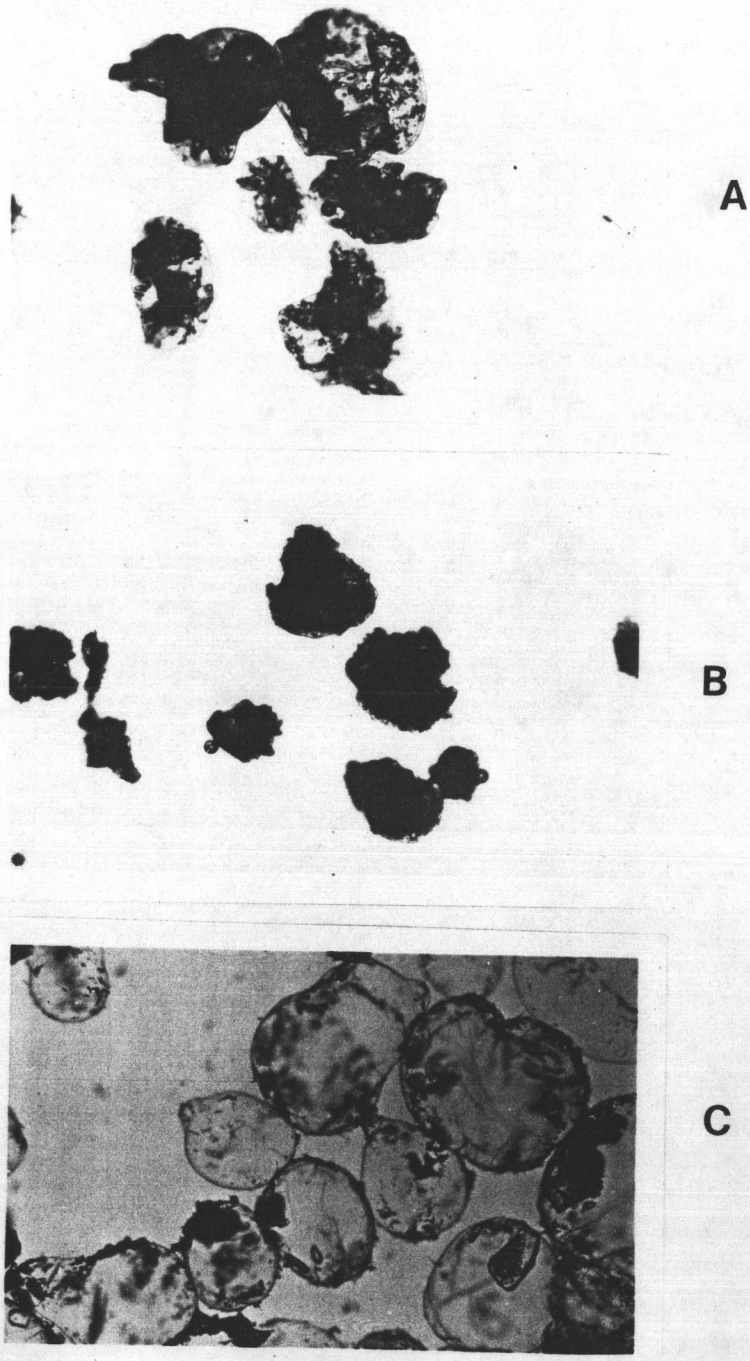


Figure 13 : Optical photomicrograph of indomethacin micocapsule prepared from glutaral 0.25 gm, 3 hr hardening time, x200 magnification

- **A : chitosan solution pH3**
- **B : chitosan solution pH4**
- **C : chitosan solution pH5**

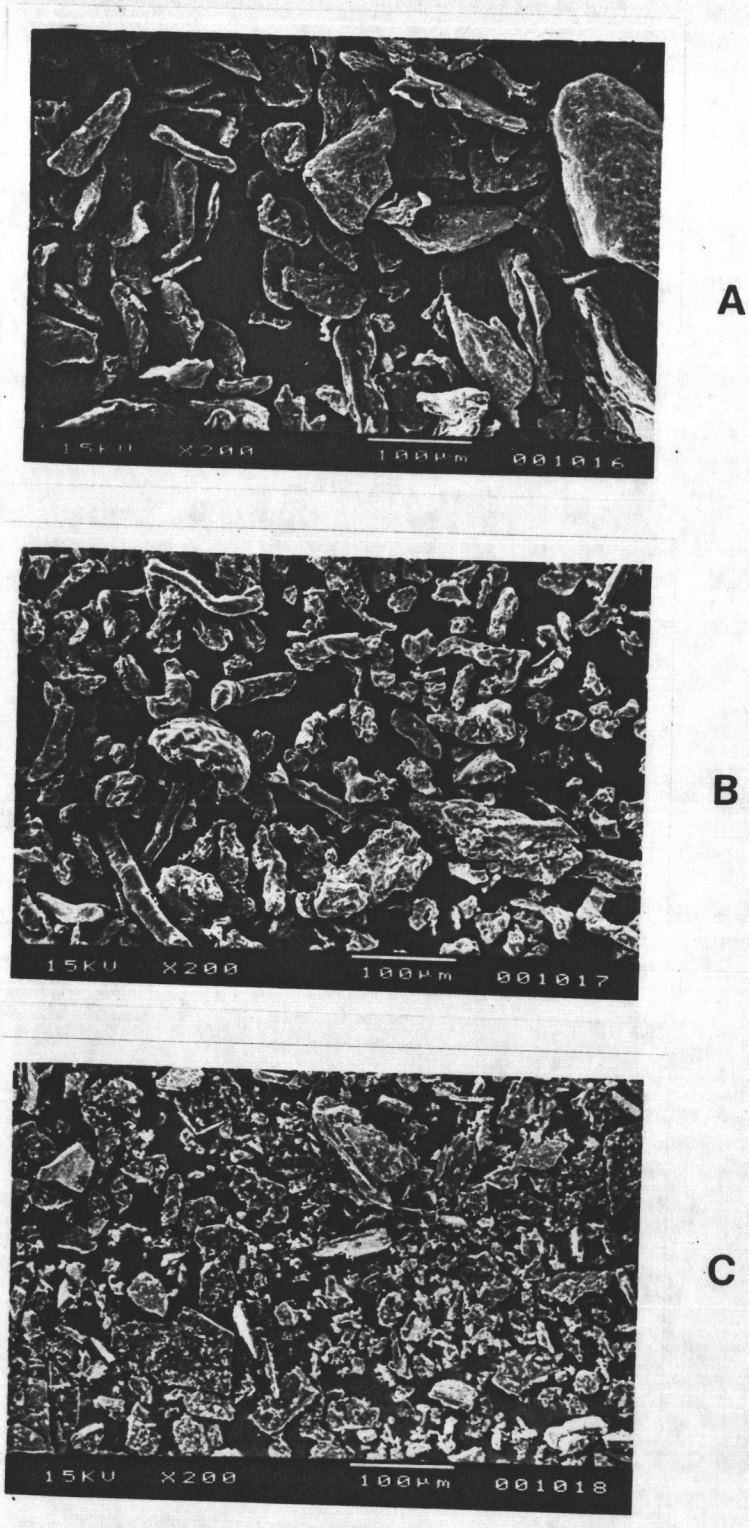


Figure 14 : Scanning electron photomicrograph of chitosan(A), CMC(B), and indomethacin (C), x200 magnification

Figures 15-17 showed the shape and surface topography of indomethacin microcapsule prepared at different pH of chitosan solution. At pH 3 of chitosan solution the folding membrane surface of the microcapsules were smoother compared with those yielded from pH 4 and pH 5 of chitosan solution. It was also notable that at this pH 3 of solution, the surface of the microcapsule had more pore on the membrane.

The surface of microcapsules from solution of pH 4 varied in roughness from smooth to heavy wavy form. In some cases some thread were found to form on the membrane surface. The roughest surface were found in those microcapsules from the solution of pH 5, in which more threads or fibers were found on the surface in a form of net like membrane with agglomerate appearance.

From all solutions, traces of indomethacin could be found on the surface of some microcapsules. The pH of chitosan solution used in the preparation seemed to have prominent effect on the morphology of the resulting microcapsules. Glutaraldehyde content and hardening time showed some effect on the morphology of the yielded microcapsules. While the concentration of chitosan solution seemed to show no significant effect on the morphology of the yielded microcapsules.

Figure 18 illustrated the shape and surface topography of indomethacin microcapsule prepared at different glutaraldehyde content using chitosan solution of pH 3 and hardening time of 3 hr. Microcapsules of glutaraldehyde 0.25 gm showed the waviest surface with some pore. While microcapsules of glutaraldehyde 2.0 gm showed the smoothest surface with some cracks on the membrane. The microcapsules started to show cracked membrane when using 1.5 gm glutaraldehyde. In addition there was a trend that the microcapsules surface became smoother but more cracked membrane when glutaraldehyde content was increased.

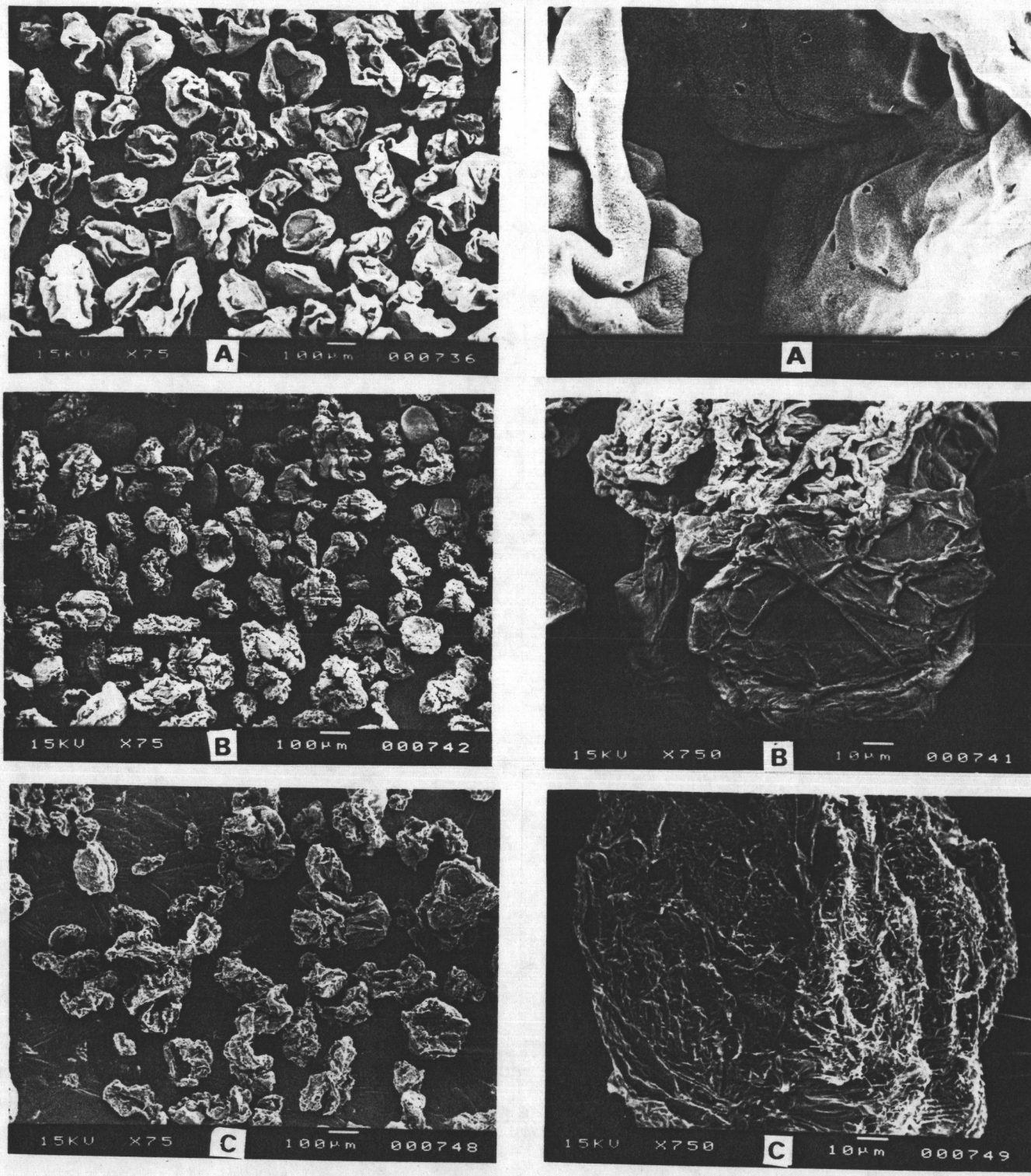


Figure 15 : Scanning electron photomicrograph of indomethacin micocapsule prepared from glutaral 0.25 gm, 3 hr hardening time, x75 and x750 magnifications

- **A : chitosan solution pH3**
- **B : chitosan solution pH4**
- **C : chitosan solution pH5**

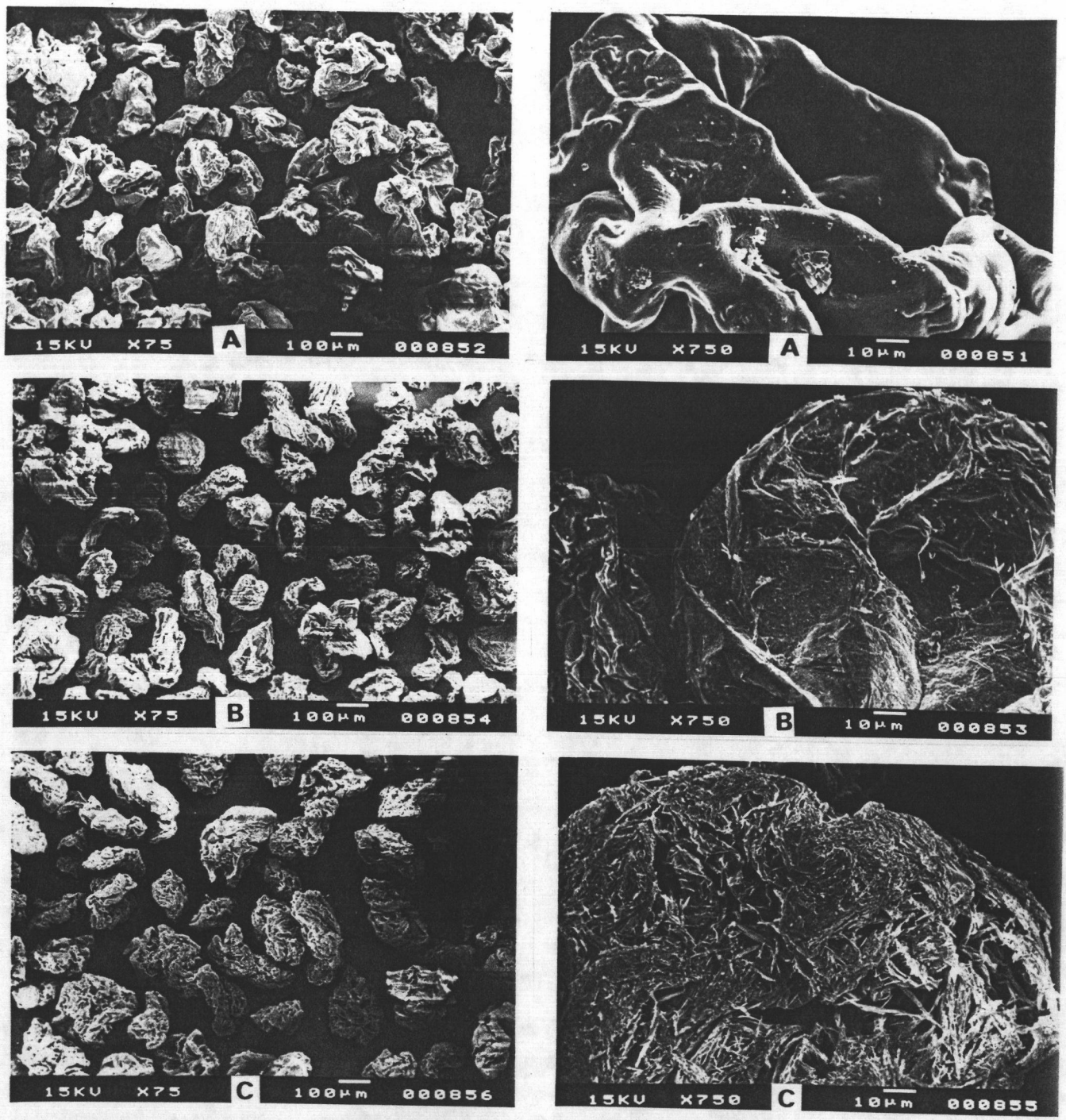


Figure 16 : Scanning electron photomicrograph of indomethacin micocapsule prepared from glutaral 0.50 gm, 3 hr hardening time, x75 and x750 magnifications

- **A : chitosan solution pH3**
- **B : chitosan solution pH4**
- **C : chitosan solution pH5**

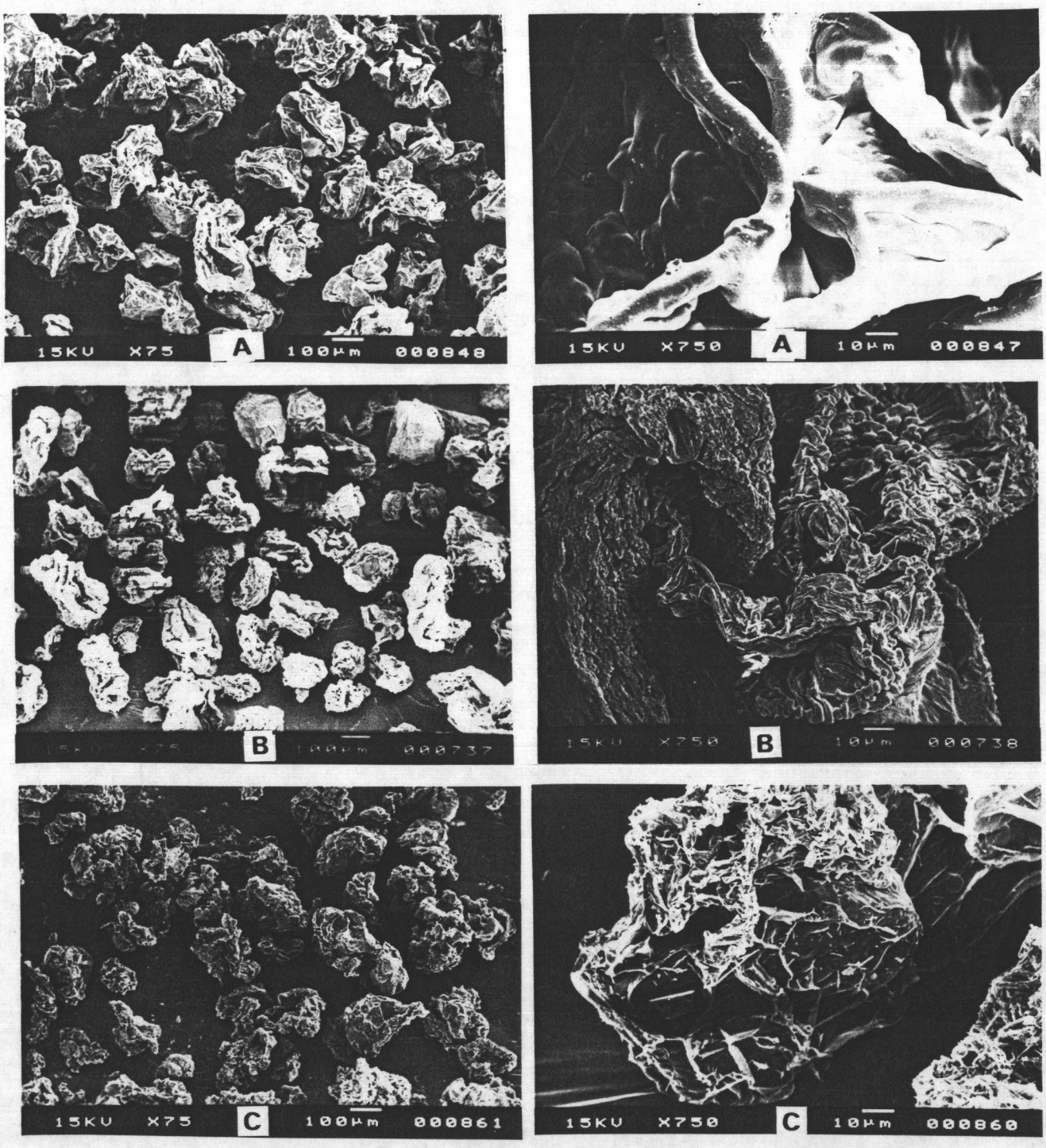


Figure 17 : Scanning electron photomicrograph of indomethacin micocapsule prepared from glutaral 1.0 gm, 1 hr hardening time, x75 and x750 magnifications

- **A : chitosan solution pH3**
- **B : chitosan solution pH4**
- **C : chitosan solution pH5**

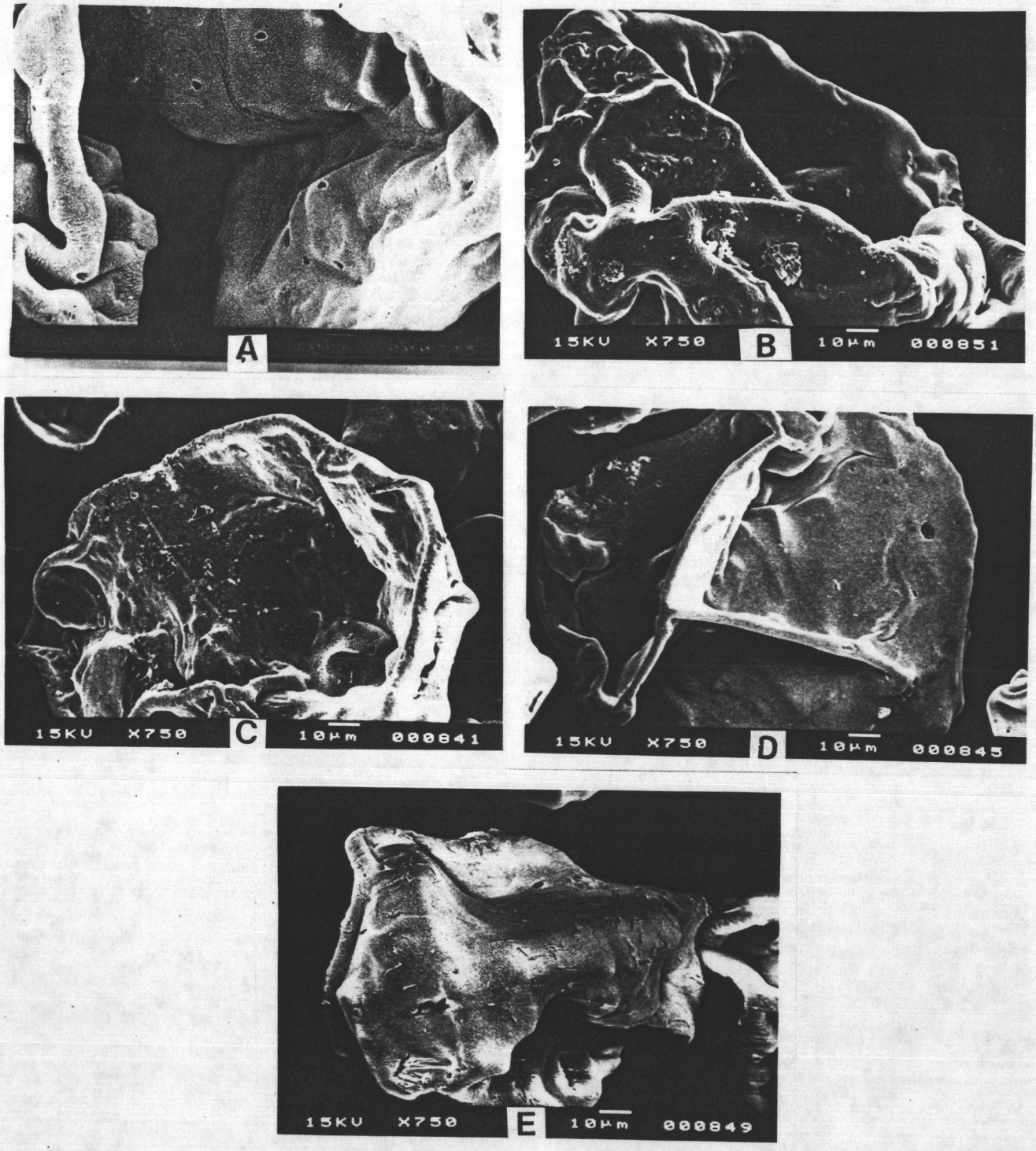


Figure 18 : Scanning electron photomicrograph of indomethacin micocapsule prepared from chitosan solution pH3, 3 hr hardening time, x750 magnification

- **A : glutaral 0.25 gm**
- **B : glutaral 0.50 gm**
- **C : glutaral 1.0 gm**
- **D : glutaral 1.5 gm**
- **E : glutaral 2.0 gm**

Figure 19 illustrated the shape and surface topography of indomethacin microcapsules prepared at different hardening time using chitosan solution of pH 3 and 1.0 gm glutaraldehyde. The surface topography of microcapsules of hardening time 1, 3, and 5 hours showed similar wavy surface. However microcapsules of the 5 hours hardening time showed some cracks on the membrane while microcapsules of the 1 hour hardening time showed more collapse. In addition microcapsules of the 1 and 5 hours hardening time showed aggregated form while microcapsule of the 3 hours hardening time showed scattered form.

Figure 20 illustrated the shape and surface topography of indomethacin microcapsules prepared at different concentration of chitosan solution using pH 4 of chitosan solution, 0.25 gm glutaraldehyde and 3 hours hardening time. All of them seemed to be not different in surface topography, which were shown creased and heavy wave form.

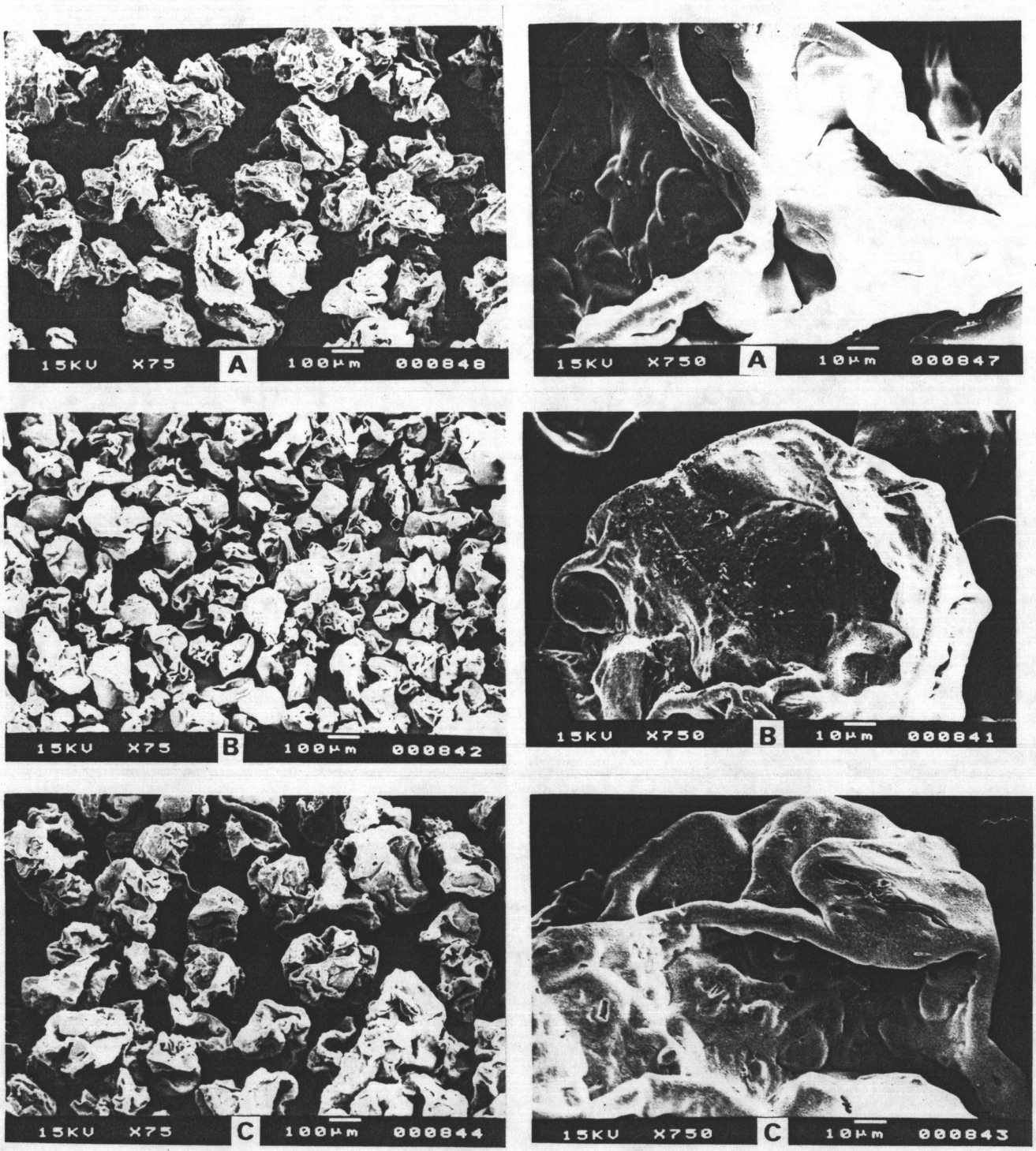
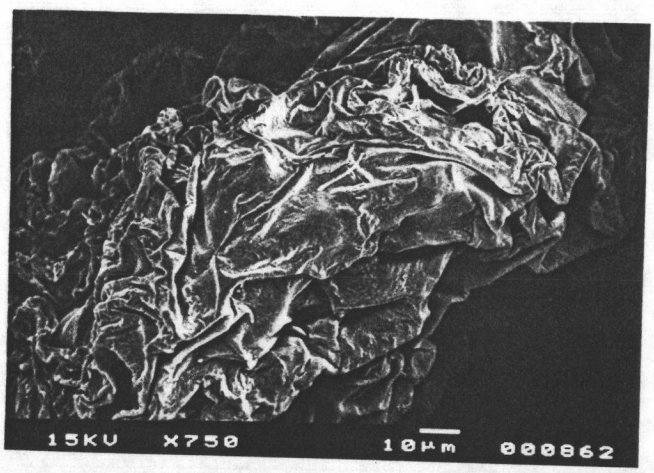
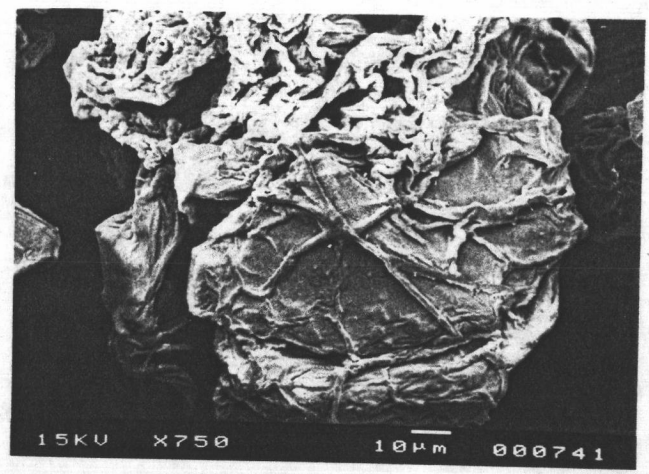


Figure 19 : Scanning electron photomicrograph of indomethacin micocapsule prepared from chitosan solution pH3, glutaral 1.0 gm , x75 and x750 magnifications

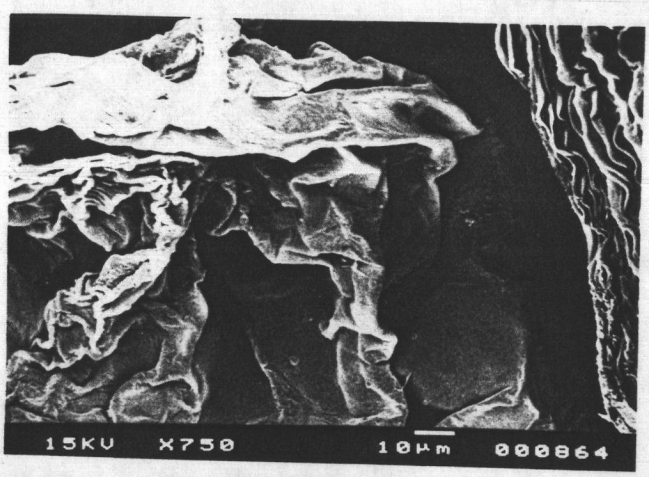
- **A : 1 hr hardening time**
- **B : 3 hr hardening time**
- **C : 5 hr hardening time**



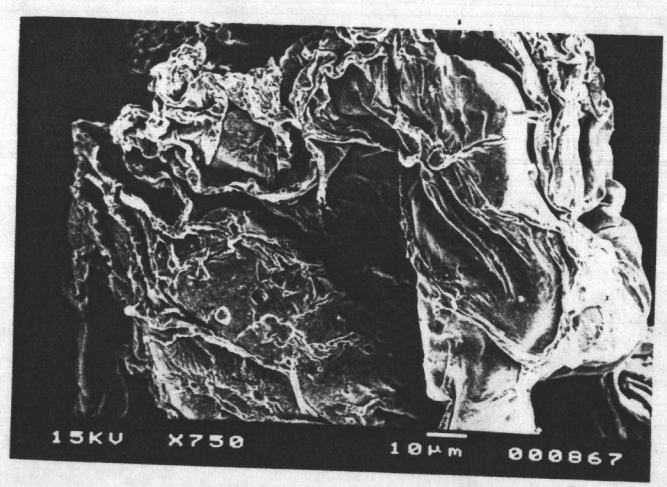
A



B



C



D

Figure 20 : Scanning electron photomicrograph of indomethacin micocapsule prepared from chitosan solution pH4, glutaral 0.25 gm, 3 hr hardening time, x750 magnification

- **A : chitosan solution 0.25 %w/v**
- **B : chitosan solution 0.50 %w/v**
- **C : chitosan solution 0.75 %w/v**
- **D : chitosan solution 1.00 %w/v**

2.2 Size and Particle Size Distribution

The number of particle size distribution and the percentage weight of particle size distribution were shown in Tables 20 and 21 in the Appendix, respectively. The cumulative percentage frequency undersize and normalised Z value (standard score), which was transferred from cumulative percentage frequency undersize were presented in Table 22 (as shown in Appendix). And geometric mean diameter at $Z = 0$, (D_{50}) of microcapsules were illustrated in Table 23 in Appendix.

2.2.1 Chitosan Solution pH

In the study of the effect of pH of chitosan solution on the particle size distribution and D_{50} of indomethacin microcapsule, three different pH of 3, 4, and 5 of chitosan solutions were used in the preparation of indomethacin microcapsules with the same glutaraldehyde content and hardening time. Figures 21 and 24 showed the comparison of % weight distribution and D_{50} respectively, with glutaraldehyde 0.25 gm and 3 hour hardening time. For Figures 22 and 25 the glutaraldehyde content used was varied to 0.50 gm and 3 hours hardening time. And Figures 23 and 26 were for 1.0 gm glutaraldehyde with 1 hour hardening time.

With the same hardening time of 3 hours, the change in glutaraldehyde content had no effect on the particle size distribution of the three pH of 3, 4, and 5 of chitosan solutions, as shown in Figures 21 and 22, the frequency distribution curve showed similar pattern at all pH of chitosan solution. With lower hardening time of 1 hour, in Figure 23, the size distribution of microcapsules of pH 3 solution showed noticeably wider distribution in the range of 47.50-388.50 microns while those microcapsules of pH 4 and pH 5 solution showed narrow distribution in the range of 47.50-295.50 microns.

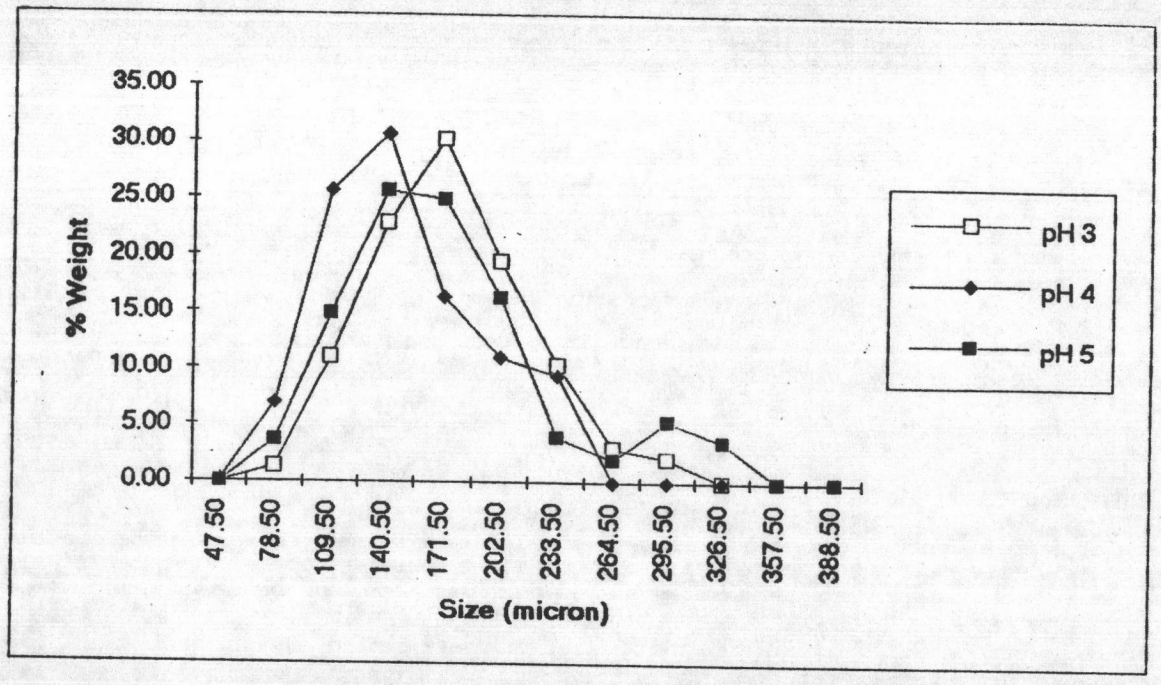


Figure 21 : The effect of chitosan solution pH on frequency curve of indomethacin microcapsule prepared from glutaral 0.25 gm and 3 hr hardening time

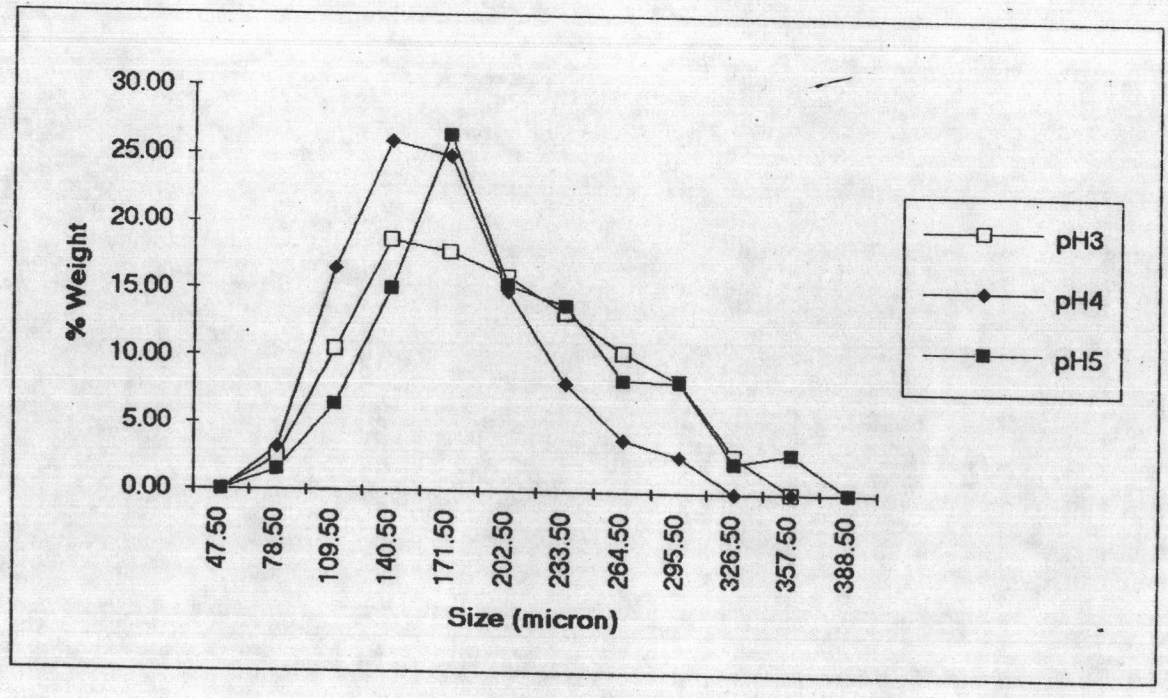


Figure 22 : The effect of chitosan solution pH on frequency curve of indomethacin microcapsule prepared from glutaral 0.50 gm and 3 hr hardening time

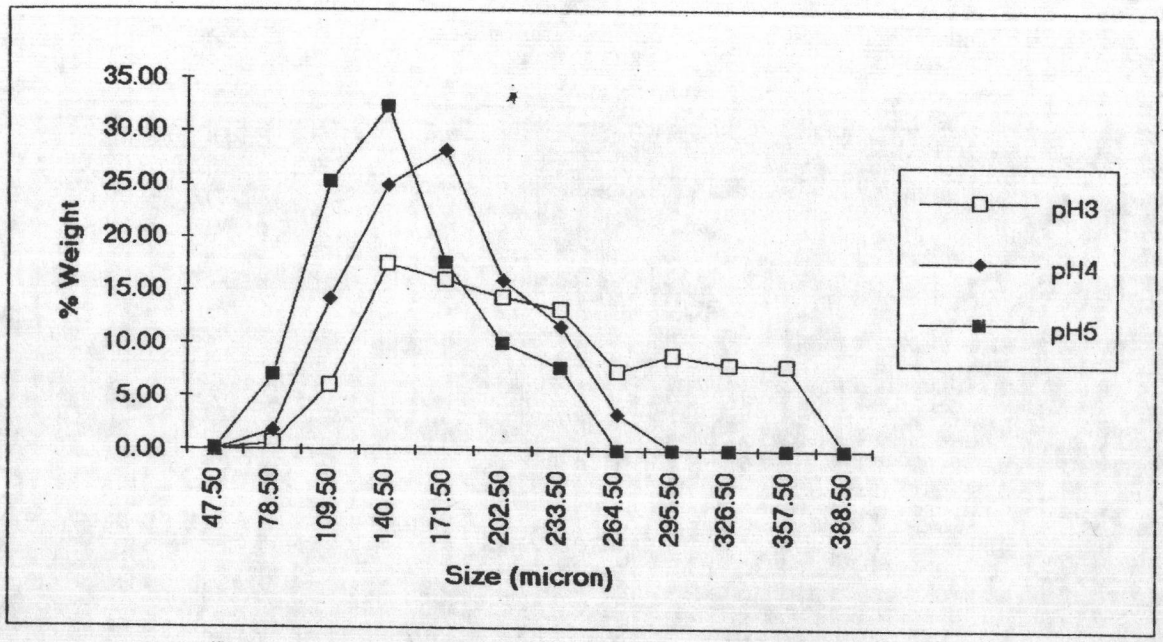


Figure 23 : The effect of chitosan solution pH on frequency curve of indomethacin microcapsule prepared from glutaral 1.0 gm and 1 hr hardening time

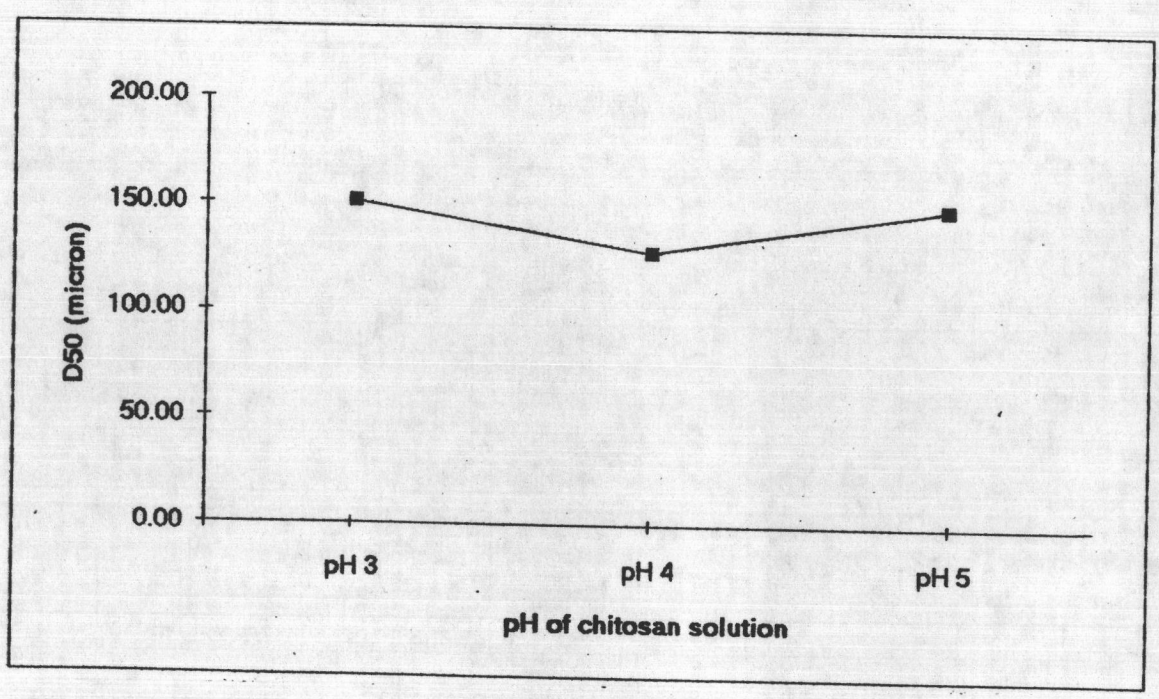


Figure 24 : The Effect of chitosan solution pH on D_{50} value of indomethacin microcapsule prepared from glutaral 0.25 gm and 3 hr hardening time

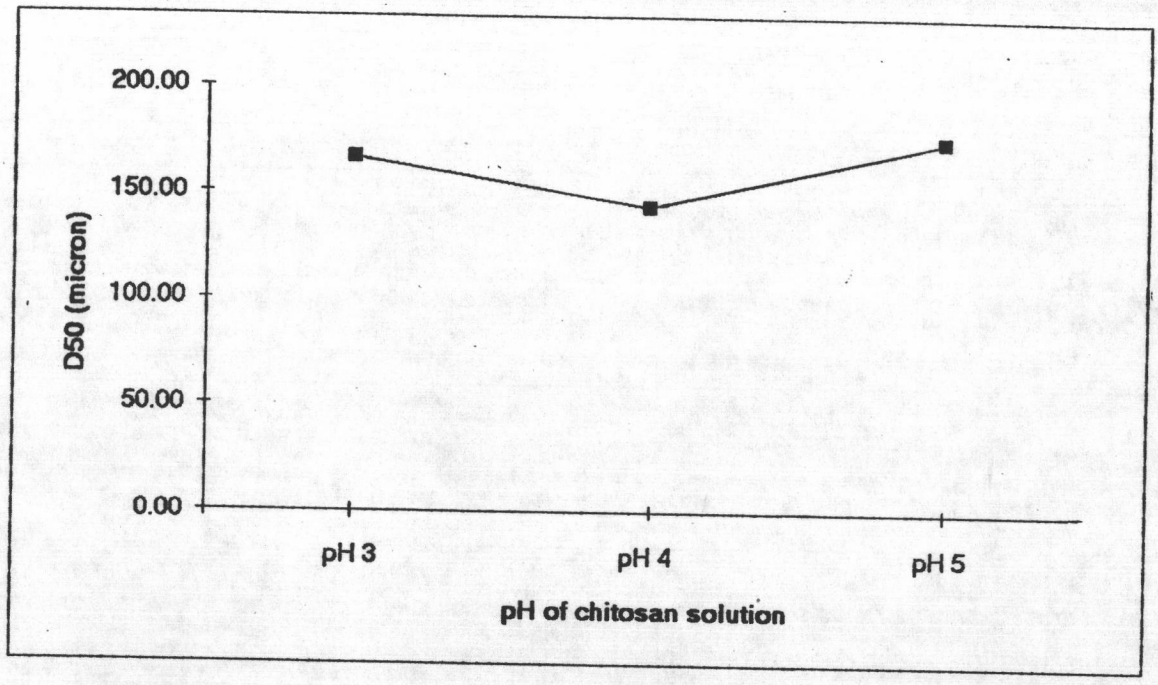


Figure 25 : The effect of chitosan solution pH on D_{50} value of indomethacin microcapsule prepared from glutaral 0.50 gm and 3 hr hardening time

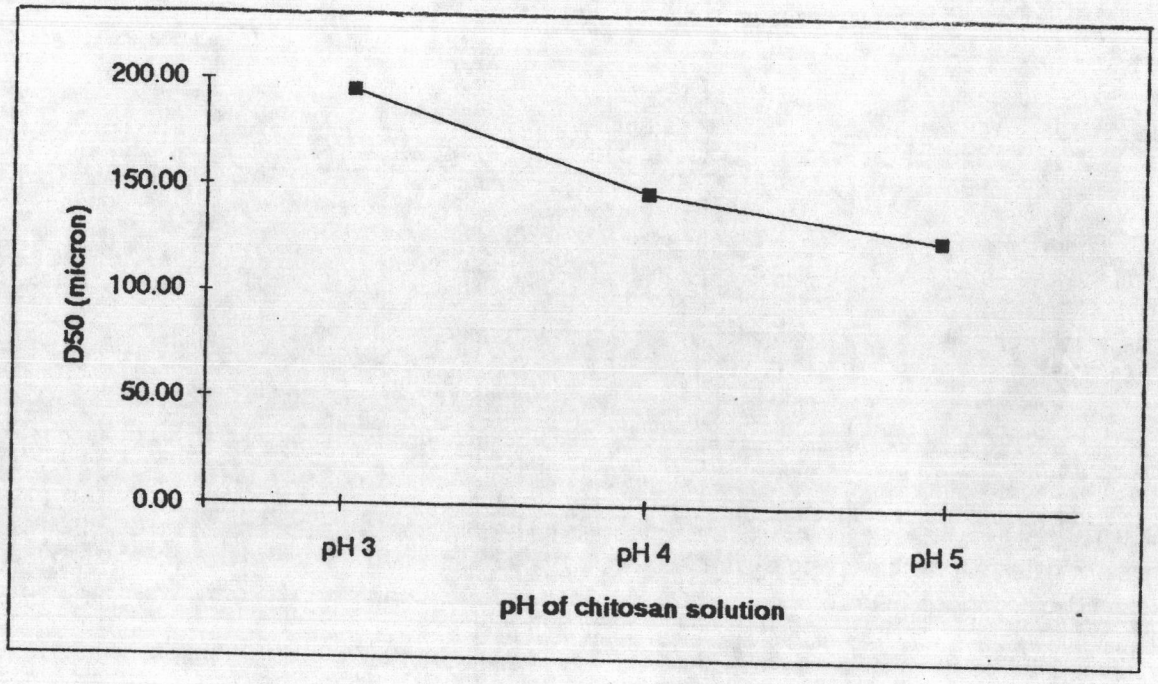


Figure 26 : The effect of chitosan solution pH on D_{50} value of indomethacin microcapsule prepared from glutaral 1.0 gm and 1 hr hardening time

Figures 24 and 25 showed the same result that chitosan solution of pH 4 gave the lowest mean value D_{50}. While Figure in 26, with the glutaraldehyde content of 1.0 gm and 1 hr hardening time, the chitosan solution of pH 5 gave the lowest D_{50} value, and the tendency was increased in particle size from 125 to 200 microns with decreasing the pH of chitosan solution.

2.2.2 Hardening Time

Figures 27 and 30 showed the effect of hardening time on size distribution and D_{50} value of microcapsules respectively, the microcapsules were prepared with glutaraldehyde 1.0 gm, and chitosan solution of pH 3. In Figures 28 and 31 the glutaraldehyde content used were 0.5 gm and pH 4 of chitosan solution. In the Figures 29 and 32, 0.5 gm glutaraldehyde and chitosan solution of pH 5 were used.

Figures 27-29 illustrated the frequency curves of different hardening times of microcapsules. Each of them showed similar pattern. In the cases of microcapsules prepared with chitosan solution of pH 3 and pH 5, with the particle size varied in the range of 47.50-388.50 microns, as shown in Figures 27 and 29. While in the case of microcapsules prepared with chitosan solution of pH 4, Figure 28, the frequency curves showed a slight different with particle size distributed in the range of 47.50-326.50 microns. However all of them showed that at 3 hours hardening time, the particle size was smaller than those with 1 hour hardening time and also 5 hour hardening time in the case of pH 3 chitosan solution. Figures 30-32 showed that at 3 hour hardening time the D_{50} value was lowest.

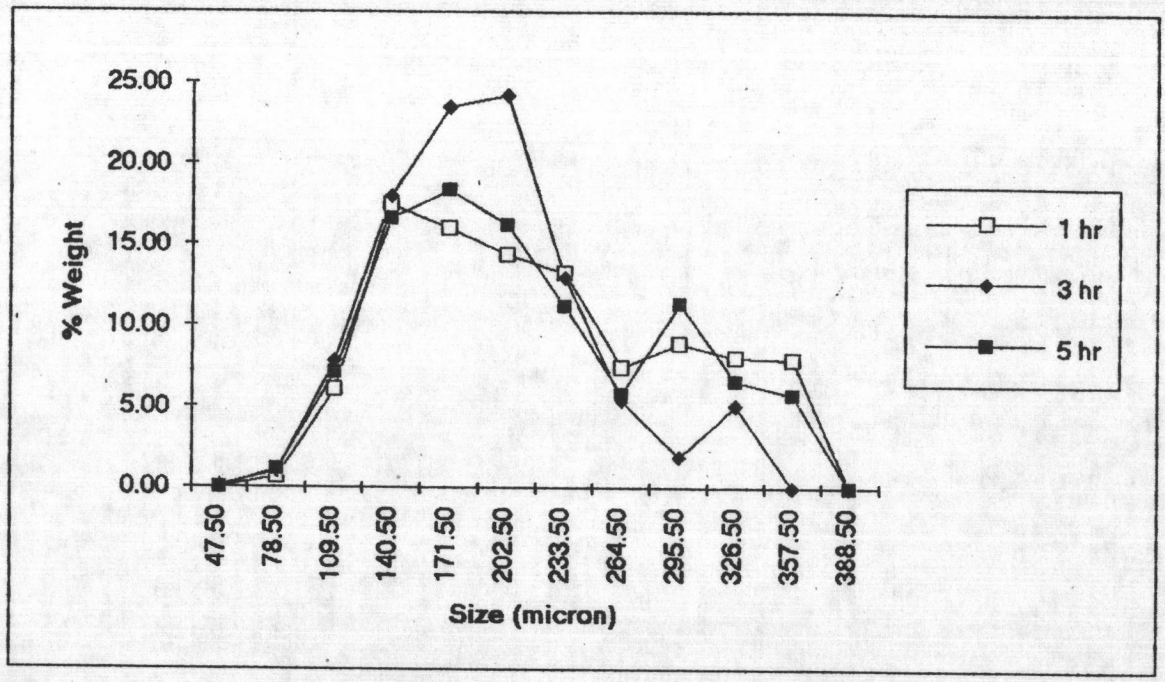


Figure 27 : The effect of hardening time on frequency curve of indomethacin microcapsule prepared from glutaral 1.0 gm, pH3 chitosan solution

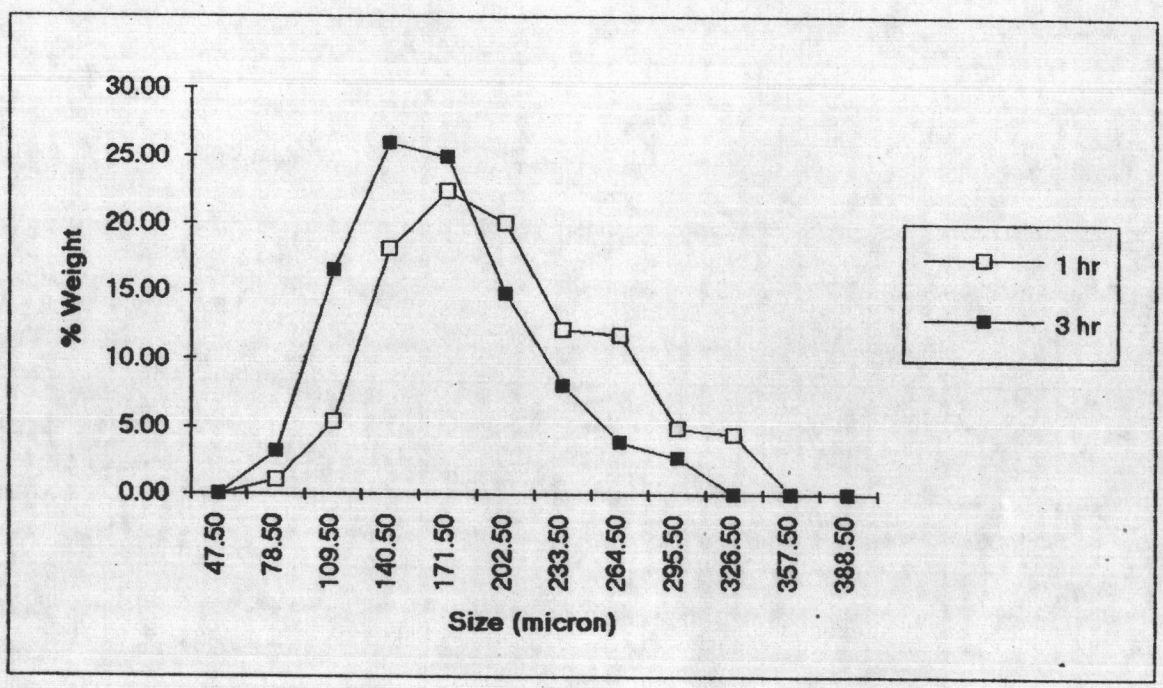


Figure 28 : The effect of hardening time on frequency curve of indomethacin microcapsule prepared from glutaral 0.5 gm, pH4 chitosan solution

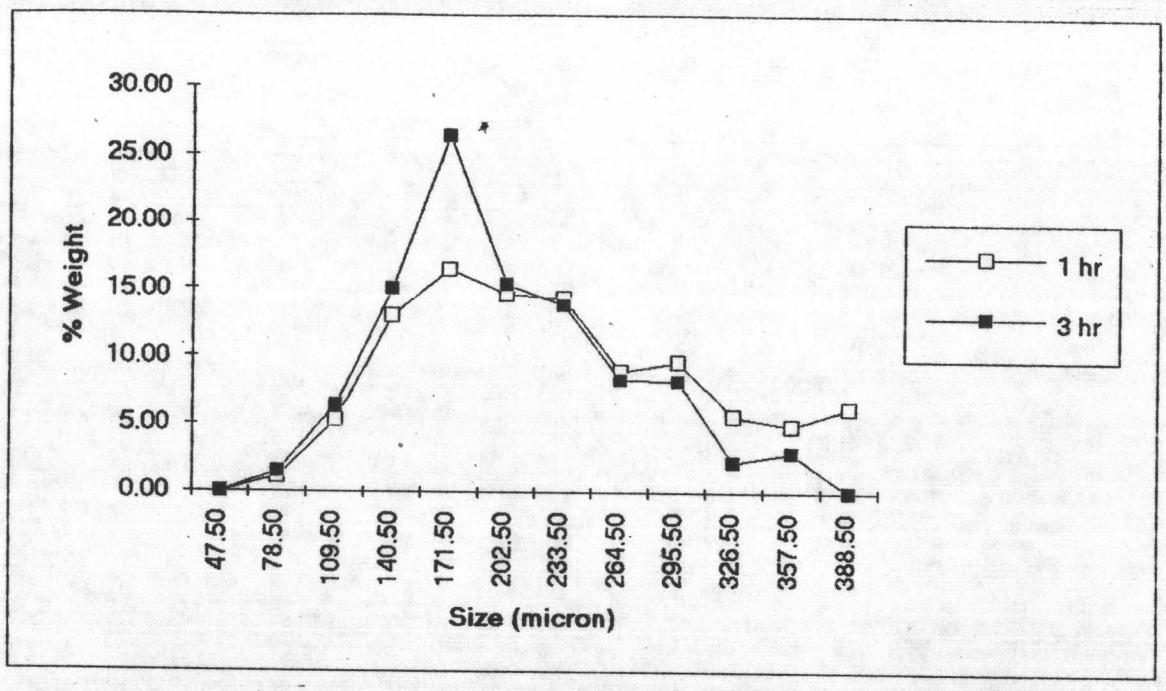


Figure 29 : The effect of hardening time on frequency curve of indomethacin microcapsule prepared from glutaral 0.5 gm, pH5 chitosan solution

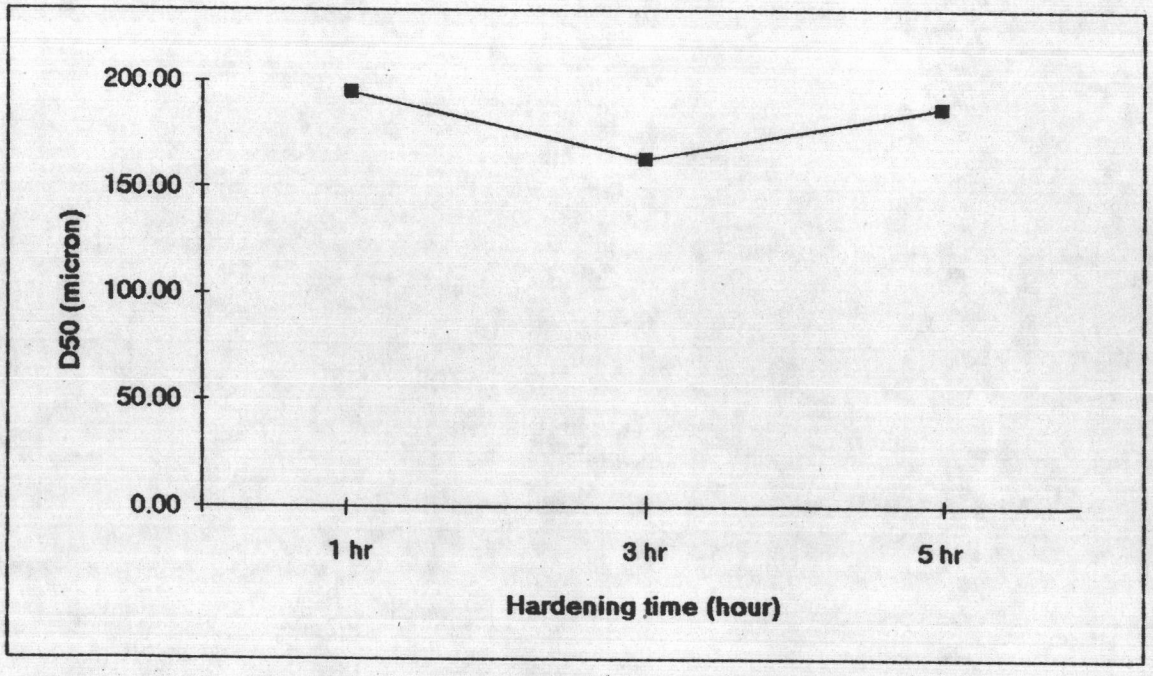


Figure 30 : The effect of hardening time on D_{50} value of indomethacin microcapsule prepared from glutaral 1.0 gm, pH3 chitosan solution

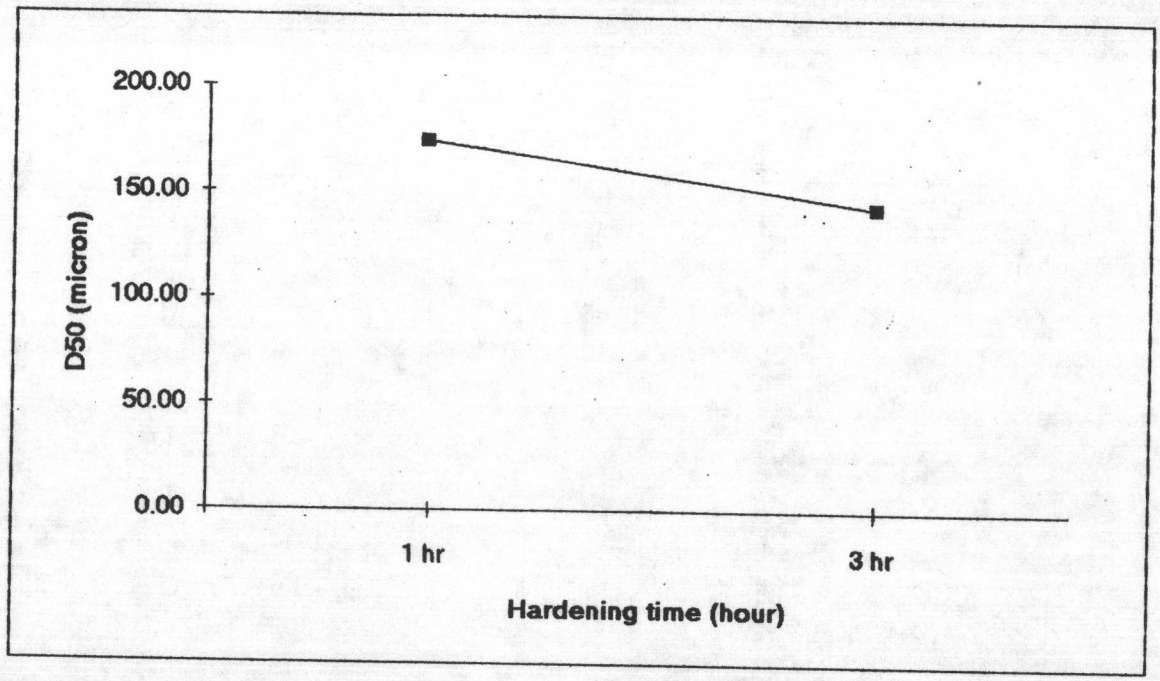


Figure 31 : The effect of hardening time on D_{50} value of indomethacin microcapsule prepared from glutaral 0.5 gm, pH4 chitosan solution

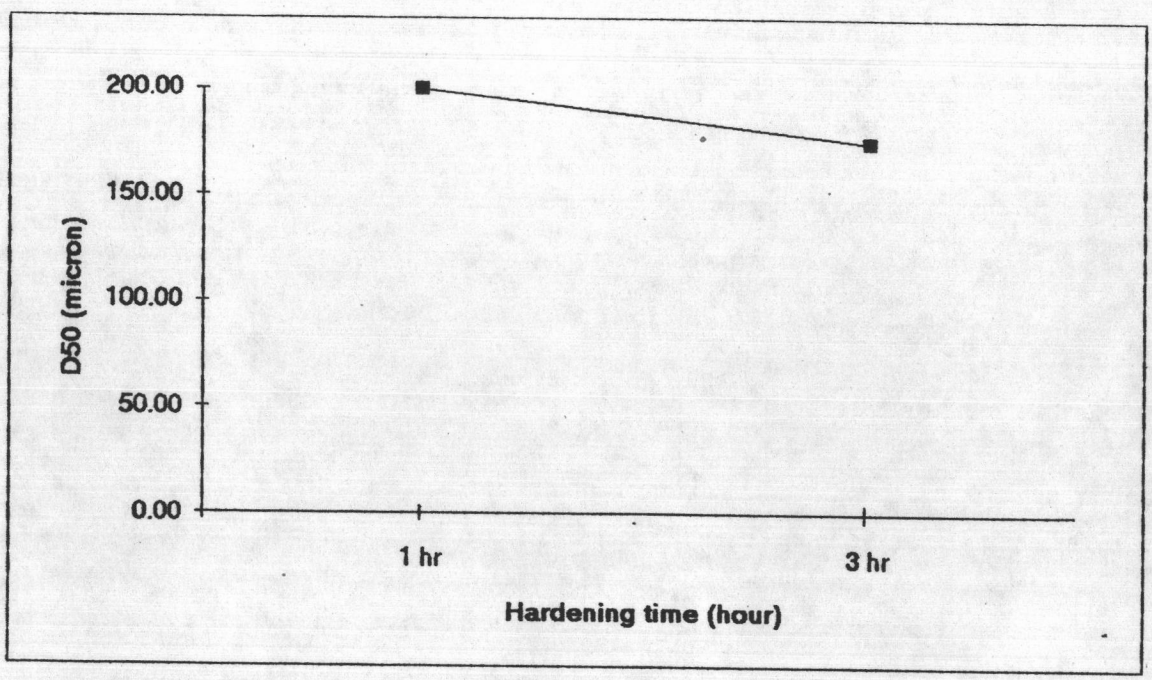


Figure 32 : The effect of hardening time on D_{50} value of indomethacin microcapsule prepared from glutaral 0.5 gm, pH5 chitosan solution

2.2.3 Glutaraldehyde Content

Figures 33-35 showed the effect of glutaraldehyde on size distribution of microcapsule prepared with the same hardening time of 3 hour and used chitosan solution of pH 3, pH 4 and pH 5 respectively. While Figures 36-38 were prepared with the same condition, but showed the effect of glutaraldehyde on D_{50} value instead of size distribution.

The results shown in the above mentioned Figures, for 3 hour hardening time, suggested the particle size distribution of microcapsules widen with increase the amount of glutaraldehyde used in the preparation. Size distribution was noticeably most narrow with low glutaraldehyde content of 0.25 gm, Figures 33-35. The D_{50} value was observed to also increased with the increased amount of glutaraldehyde content in the cases of microcapsule prepared from pH 4 and pH 5 of chitosan solution, Figures 37-38. While microcapsules of pH 3 solution showed no marked different, Figure 36. And the above mention Figures showed that lowest D_{50} value occurred at 0.25 gm glutaraldehyde.

Figures 39-40 showed the effect of glutaraldehyde on size distribution of microcapsule prepared with the same hardening time of 1 hour and used chitosan solution of pH 4 and pH 5 respectively. While Figures 41-42 were prepared with the same condition, but showed the effect of glutaraldehyde on D_{50} value instead of size distribution.

With the hardening time of 1 hour, size distribution behaviour somewhat different from the previous cases. With pH 4 of chitosan solution the frequency curve showed no different between the different amount of glutaraldehyde used, Figure 39. While with pH 5 of chitosan solution, Figure 40, there was more larger microcapsules in the case of 0.5 gm glutaraldehyde than that of 1.0 gm glutaraldehyde. Figures 41 and 42 concurred that lowest D_{50} value occurred at 1.0 gm glutaraldehyde.

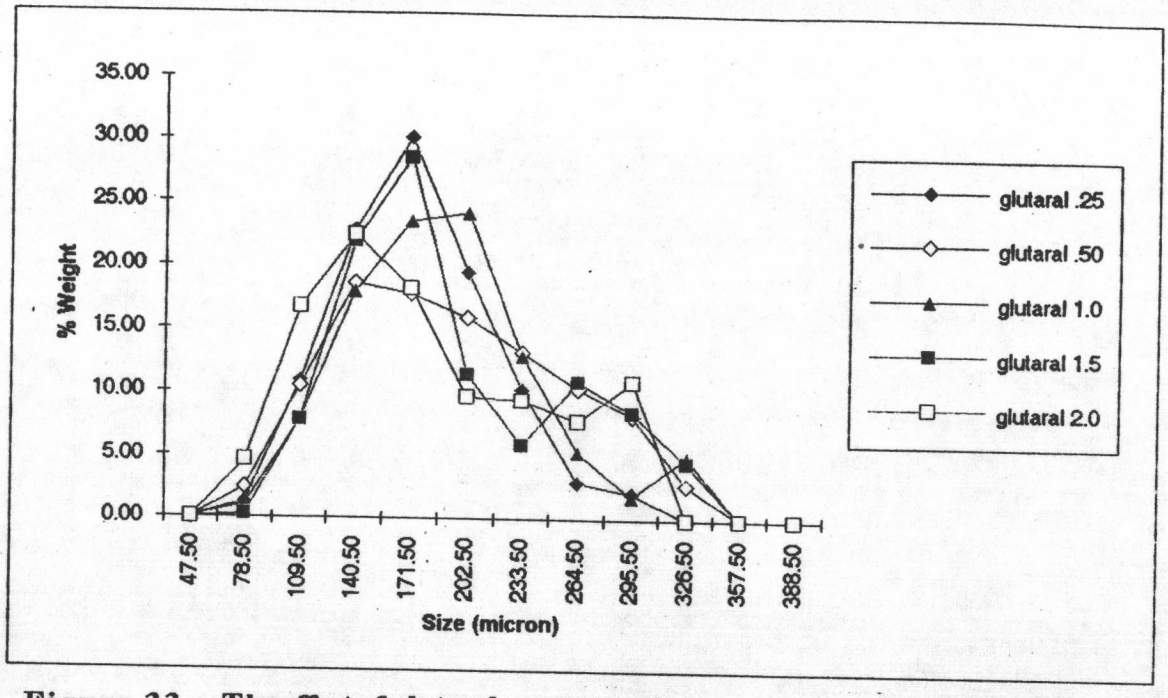


Figure 33 : The effect of glutaral content on frequency curve of indomethacin microcapsule prepared from 3hr hardening time, pH3 chitosan solution

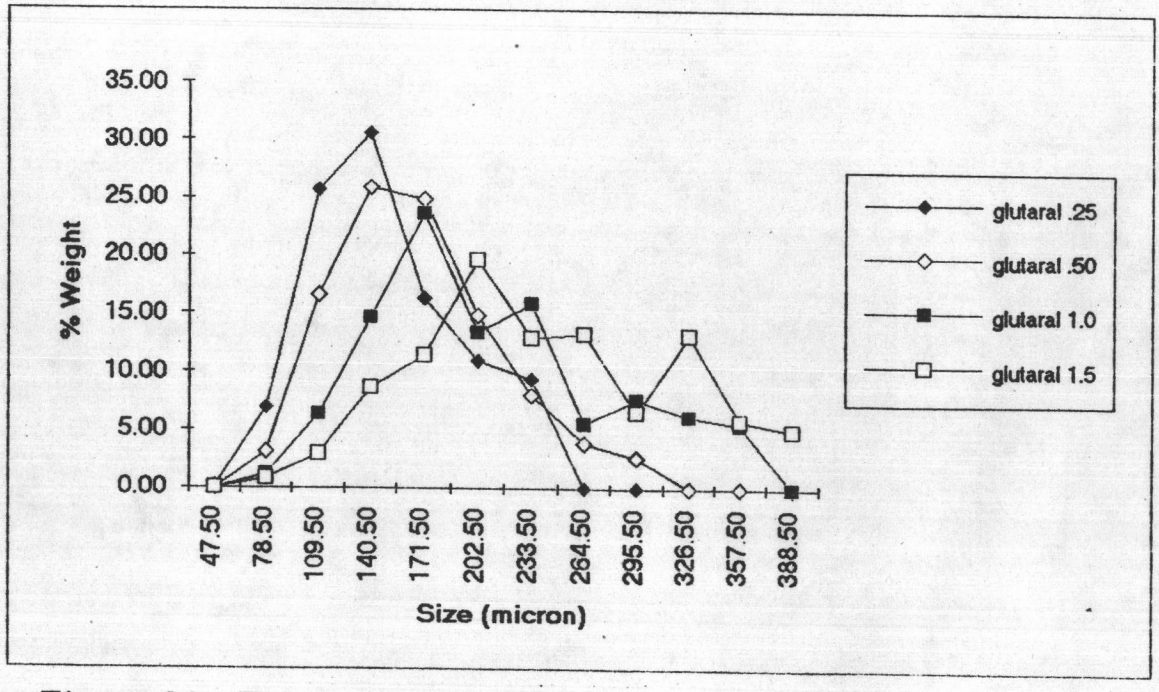


Figure 34 : The effect of glutaral content on frequency curve of indomethacin microcapsule prepared from 3hr hardening time, pH4 chitosan solution

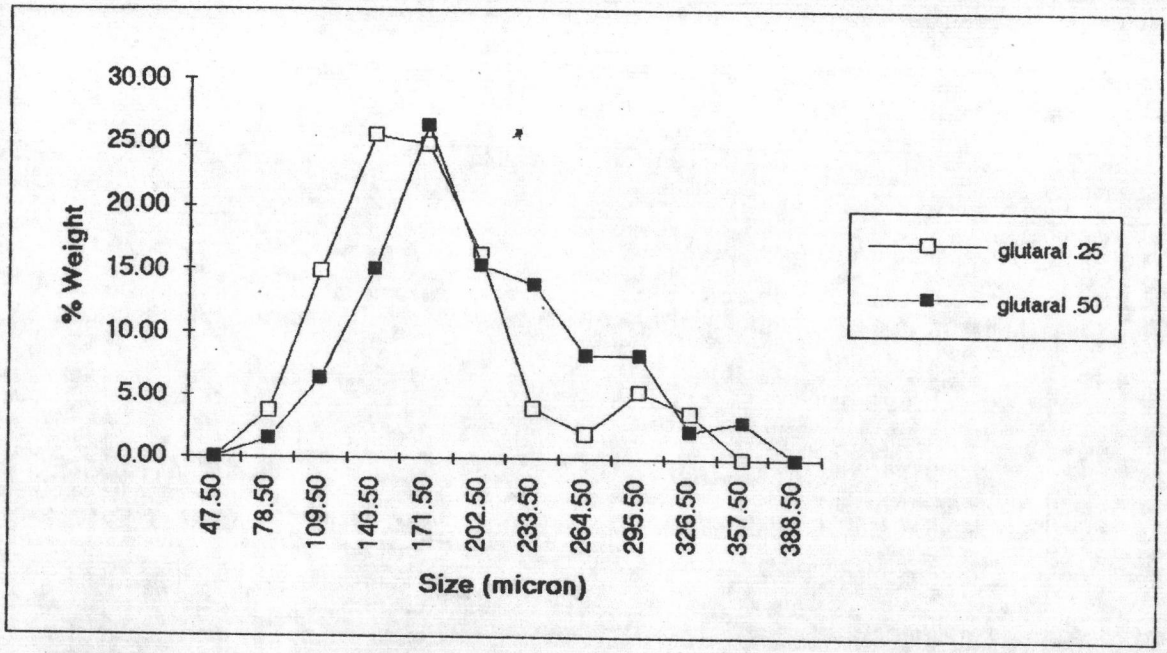


Figure 35 : The effect of glutaral content on frequency curve of indomethacin microcapsule prepared from 3hr hardening time, pH5 chitosan solution

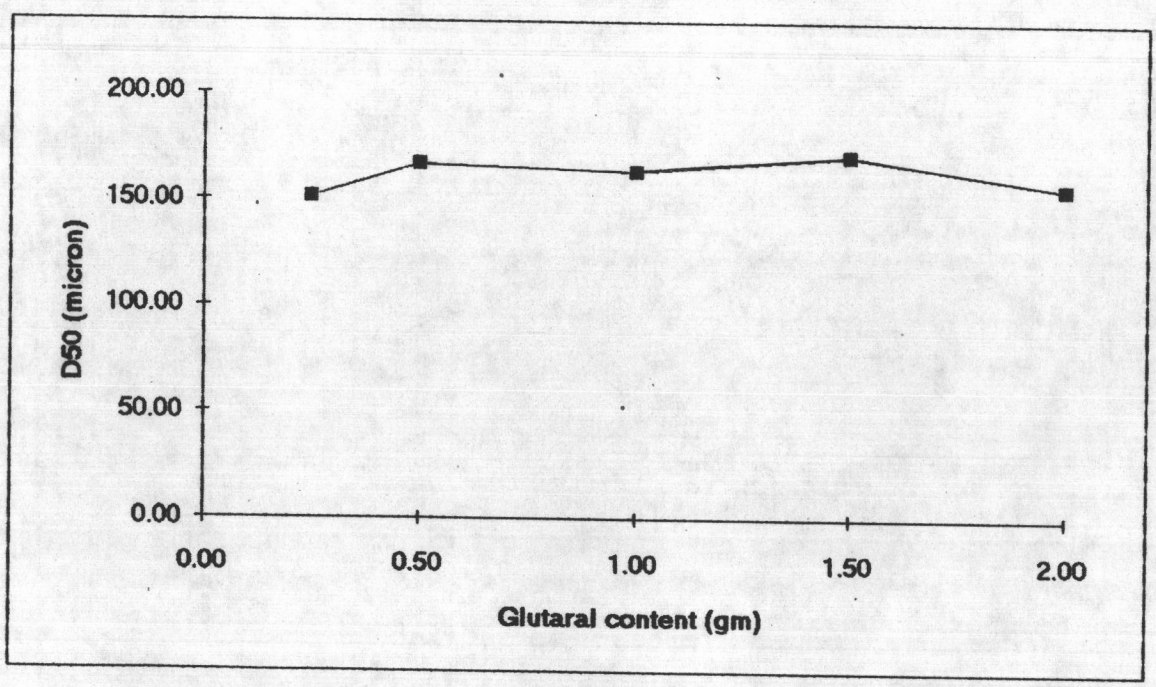


Figure 36 : The effect of glutaral content on D_{50} value of indomethacin microcapsule prepared from 3hr hardening time, pH3 chitosan solution

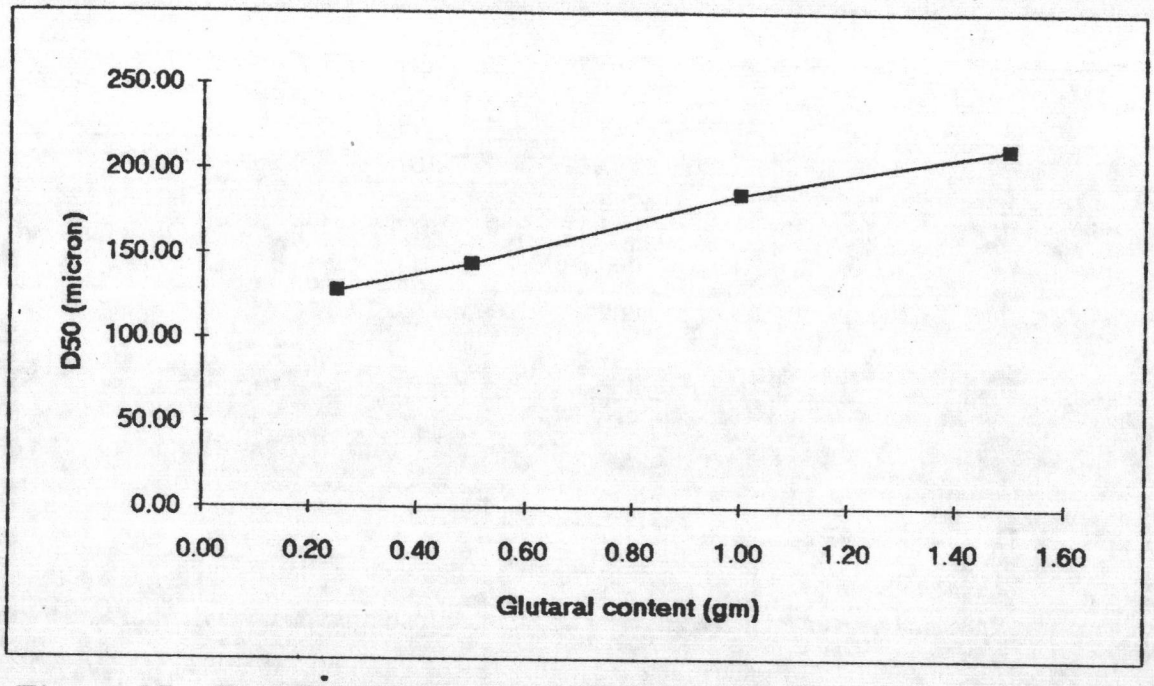


Figure 37 : The effect of glutaral content on D_{50} value of indomethacin microcapsule prepared from 3hr hardening time, pH4 chitosan solution

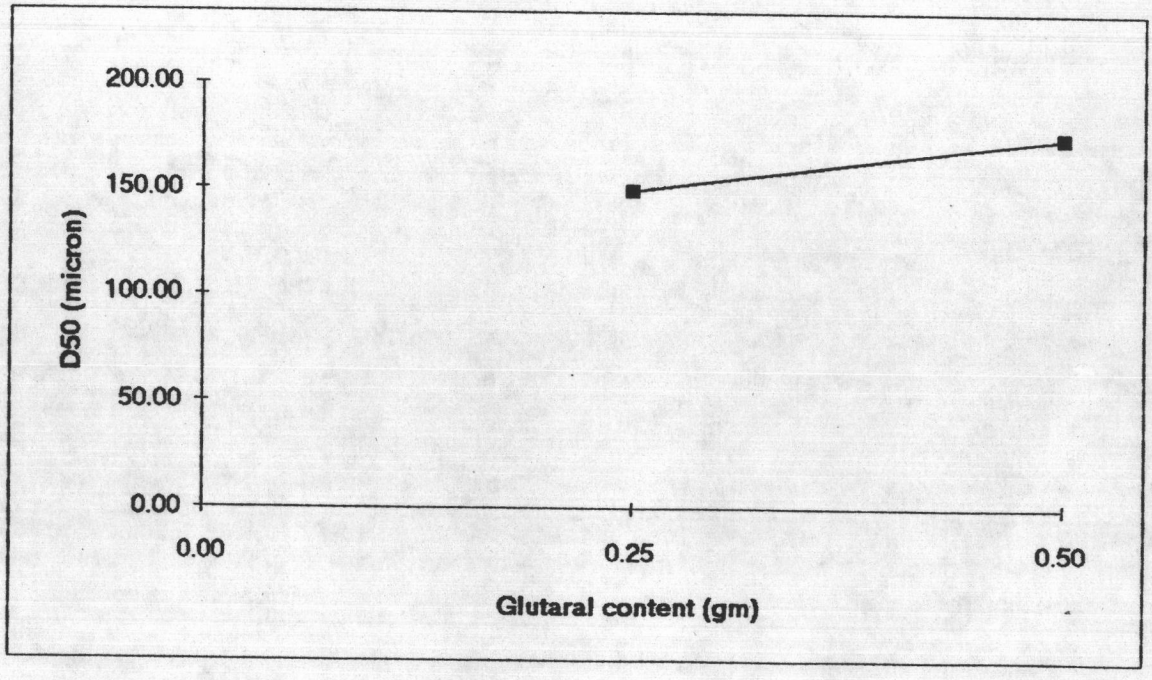


Figure 38 : The effect of glutaral content on D_{50} value of indomethacin microcapsule prepared from 3hr hardening time, pH5 chitosan solution

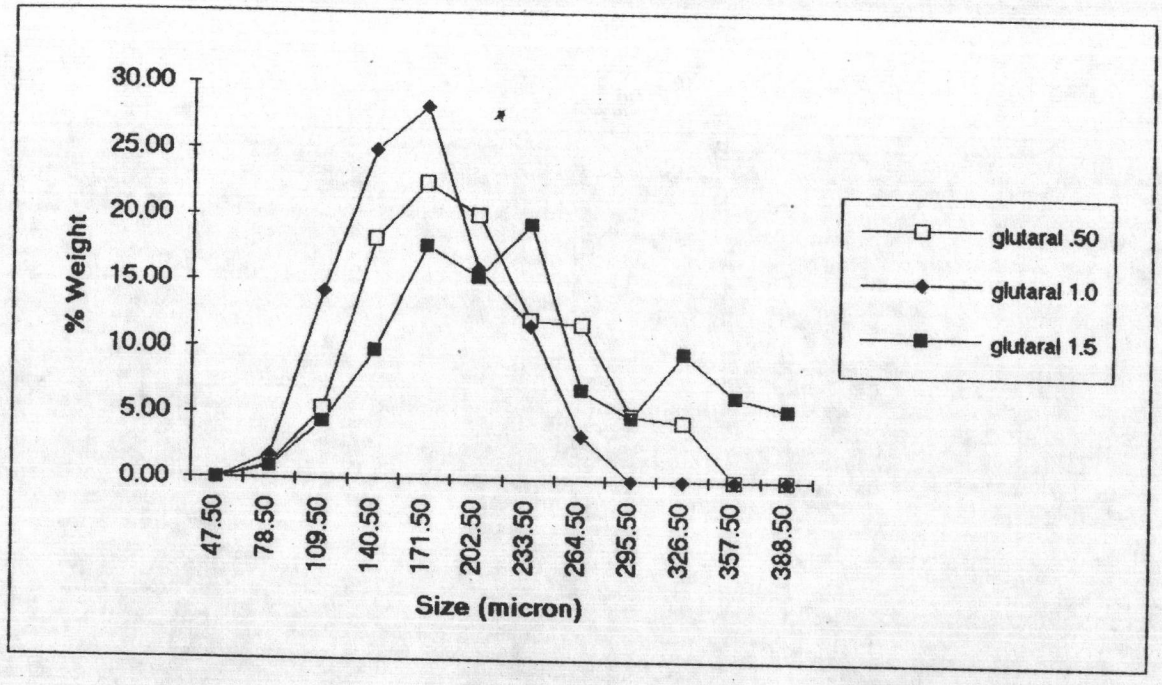


Figure 39 : The effect of glutaral content on frequency curve of indomethacin microcapsule prepared from 1hr hardening time, pH4 chitosan solution

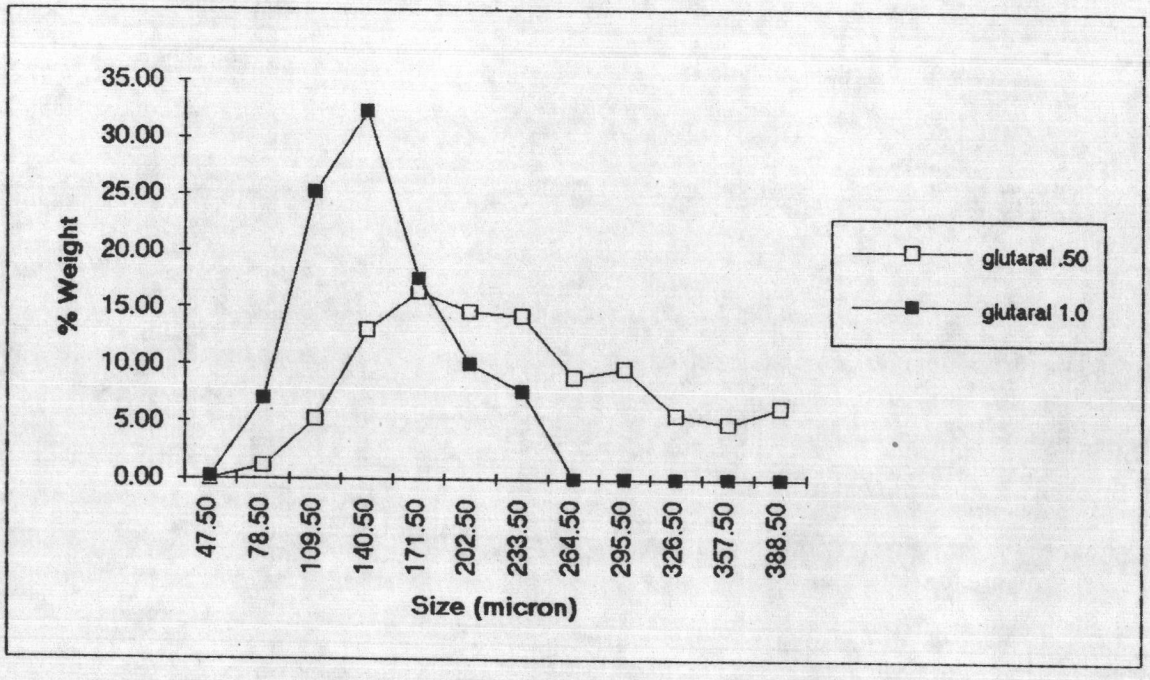


Figure 40 : The effect of glutaral content on frequency curve of indomethacin microcapsule prepared from 1 hr hardening time, pH5 chitosan solution

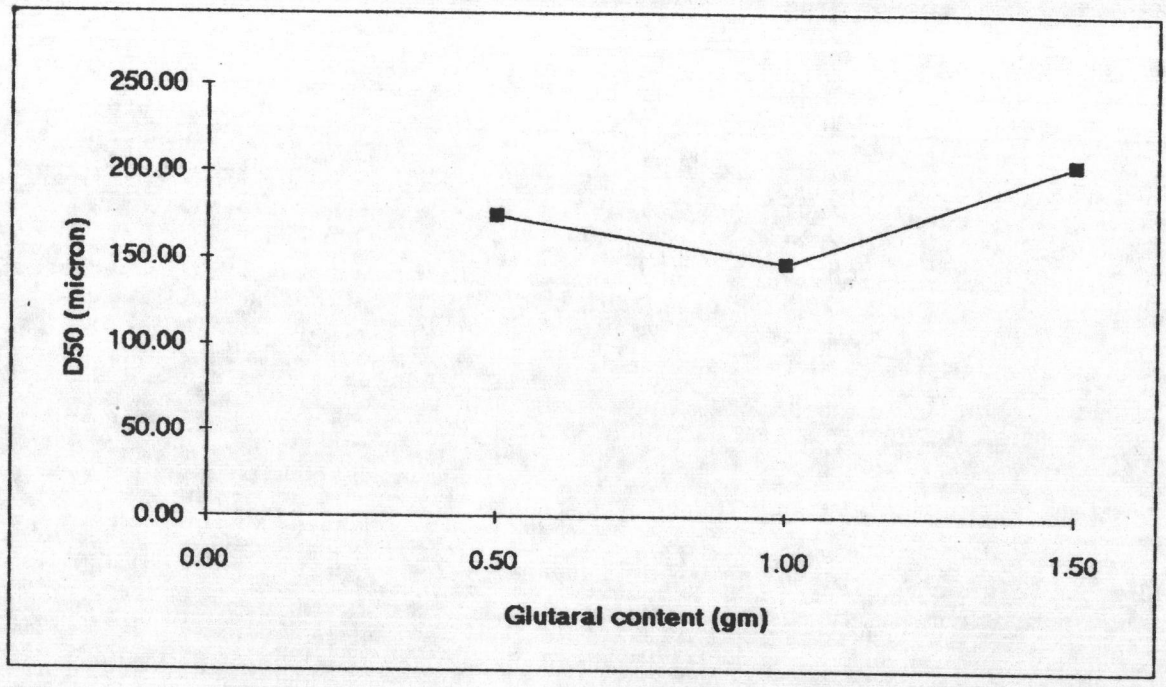


Figure 41 : The effect of glutaral content on D_{50} value of indomethacin microcapsule prepared from 1 hr hardening time, pH4 chitosan solution

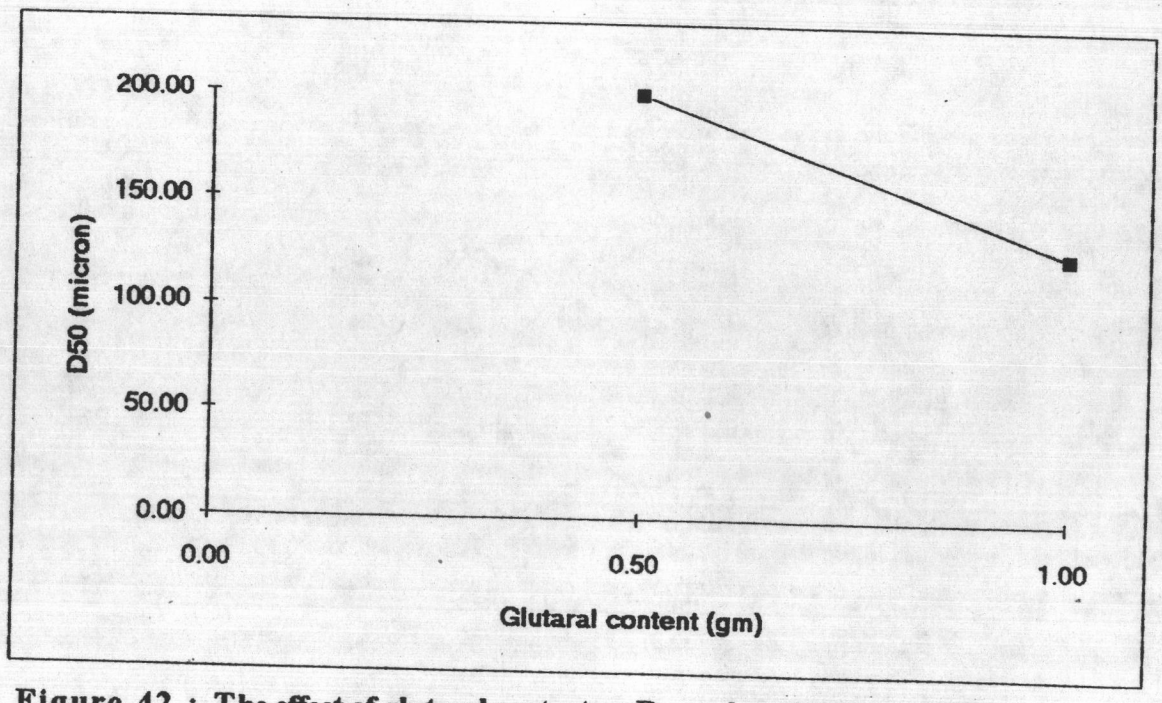


Figure 42 : The effect of glutaral content on D_{50} value of indomethacin microcapsule prepared from 1 hr hardening time, pH5 chitosan solution

2.2.4 Chitosan solution Concentration

Figures 43 and 44 showed the effect of chitosan solution concentration on size distribution and D_{50} value of indomethacin microcapsule respectively. The microcapsules were prepared with chitosan solution of pH 4, hardening time 3 hours and 0.25 gm glutaraldehyde content.

Figure 43 showed slight different pattern of frequency curve of various chitosan solution concentrations. Microcapsules prepared from 0.5%w/v chitosan solution showed narrower size distribution, in the range of 47.50-264.50 microns, than microcapsule from other concentration of chitosan solutions and had smaller size. In Figure 44 the D_{50} value was greatest at chitosan concentration of 0.25%w/v while the size was smallest when the chitosan solution was 0.5%w/v, and there were no different in size for the concentration of 0.75% and 1.0%w/v.

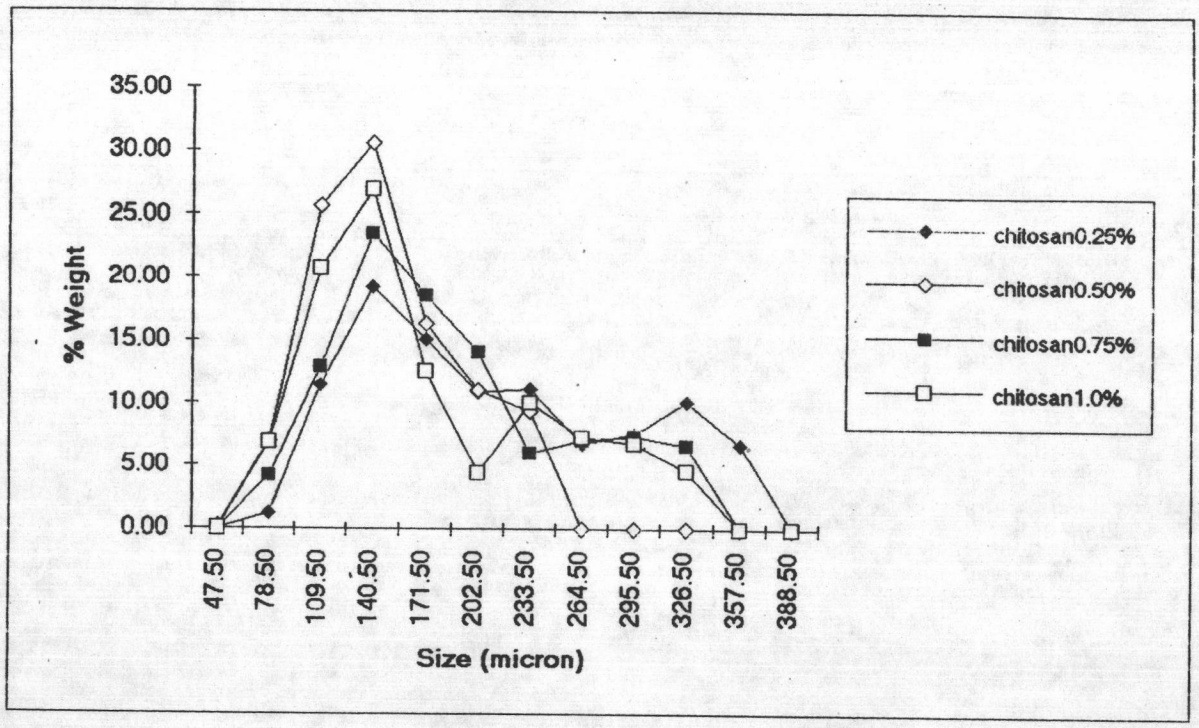


Figure 43 : The effect of chitosan concentration on frequency curve of indomethacin microcapsule prepared from pH4 chitosan solution, 0.25 gm glutaral and 3hr hardening time.

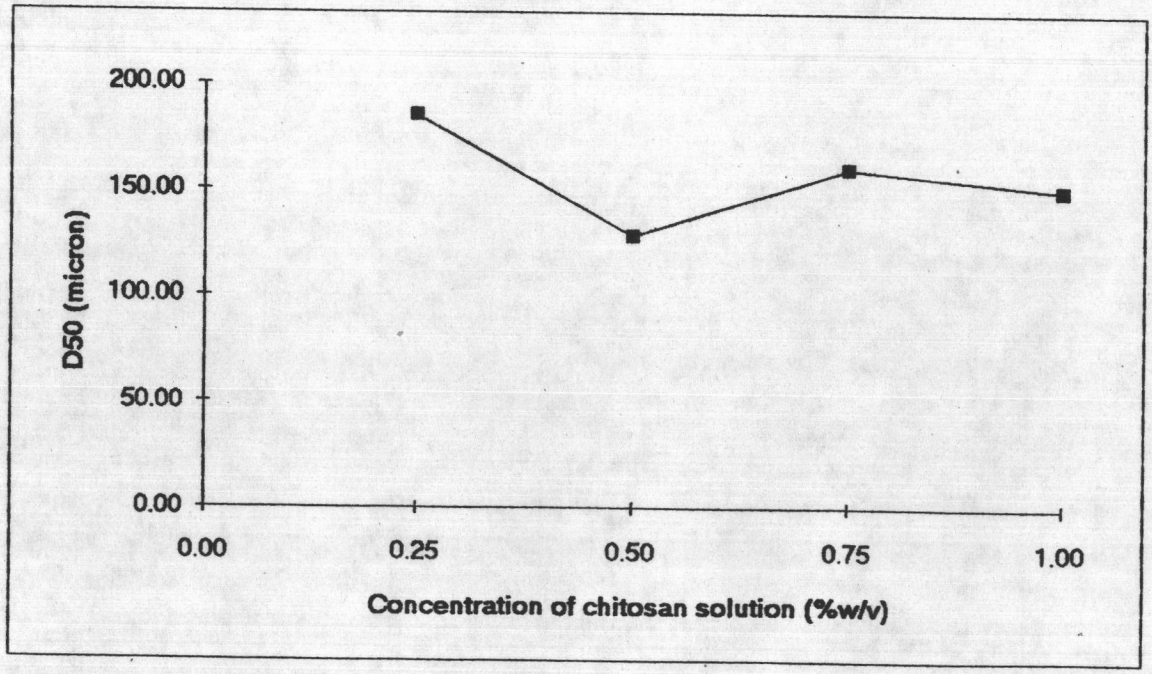


Figure 44 : The effect of chitosan concentration on D_{50} value of indomethacin microcapsule prepared from pH4 chitosan solution, 0.25 gm glutaral and 3hr hardening time

2.3 Drug Entrapment and Drug Recovery

Indomethacin microcapsules prepared by complex coacervation techniques showed drug entrapment in the range of 39.13 - 48.35 %, while its drug recovery ranged between 50.98 - 86.67 %. The same process failed to entrap drugs when pindolol was used as model drug in place of indomethacin. The following observations hence concerned only the influences of processing conditions on drug entrapment and drug recovery of indomethacin microcapsules.

Table 9 showed the drug entrapment and drug recovery percentage of indomethacin microcapsules prepared under different processing conditions. Preparations of 1-11, 13-19, and 21-25 were prepared from chitosan solution of pH 3, pH 4, and pH 5 respectively with different hardening time and glutaraldehyde content. From the solutions of pH 3, pH 4 and pH 5, the percentage of drug entrapments were varied in the range of 40.25-45.54%, 41.12-46.54% and 41.29-48.35% respectively. From these results, it was suggested that the pH of chitosan solution seemed to have no effect on the drug entrapment of the microencapsulation process. The same results occurred with the varying hardening time on the drug entrapment of the microencapsulation process. Preparations of 13-15 and 17-19 were prepared from chitosan solution of pH 4 with 1 and 3 hours hardening time respectively. The results showed that percentage of drug entrapments were varied in the range of 42.38-46.56% and 41.12-45.38% for the hardening time of 1 and 3 hours, respectively.

The effect of glutaraldehyde content on percentage of drug entrapment seemed to be no consistent. At longer hardening time of 5 and 3 hours (preparations 1-3 and 16-19 respectively), preparations of high glutaraldehyde content showed the highest percentage of drug entrapment (preparation 3 and 19). But at hardening time of 1 hour (preparations 13-15 and 21-22), preparations of high glutaraldehyde content exhibited the lowest percentage of drug entrapment (preparations 15 and 22).

From the same table, it was also observed that the drug recovery could be influenced by the pH of chitosan solution. Preparations that were prepared with lower pH of chitosan solution achieved greater drug recovery than those prepared with higher pH of chitosan solution. The most notable results was very low drug recovery for formulation prepared with chitosan solution of pH 5 in comparison to those prepared with solution of pH 3 and pH 4.

Table 10 showed the effects of chitosan solution concentration on the percentage of drug entrapment and drug recovery. The results indicated that higher drug entrapment and drug recovery could be achieved by increasing the concentration of chitosan solutions.

Table 9 : Percentage of drug entrapment and drug recovery of indomethacin microcapsules

Preparation	% Drug Entrapment	% Drug Recovery
1	40.25	74.18
2	40.06	75.36
3	43.31	86.67
4	43.0	70.79
5	43.38	79.38
6	41.44	74.51
7	40.0	69.95
8	42.42	77.78
11	45.54	81.63
13	46.56	69.3
14	42.9	64.73
15	42.38	66.23
16	41.68	74.07
17	41.12	71.08
18	43.08	71.18
19	45.38	75.25
21	48.35	63.94
22	44.54	66.22
24	42.69	60.72
25	41.29	50.98

* Average from 3 determinations

Table 10 : Percentage of drug entrapment and drug recovery of indomethacin microcapsules prepared from different chitosan solution concentration

Preparation	Chitosan Conc. (% w/v)	% Drug Entrapment	% Drug Recovery
28	0.25	39.13	60.92
16	0.50	41.68	74.07
29	0.75	43.98	77.04
30	1.00	45.92	80.29

Average from 3 determinations

2.4 Drug Release Study

The drug release data of each preparation was described in Table 24 (in Appendix) and were shown graphically as Higuchi's plot in Figures 46-57. Each point presented the average value obtained from three samples at a given sampling time. The processing factors which affected the drug release of microcapsule were chitosan solutions pH, hardening time, glutaraldehyde content, and chitosan solution concentration. The effects of these factors were discussed in details in the following section.

2.4.1 Chitosan Solution pH

The comparison were made for indomethacin microcapsule prepared from chitosan solution of pH 3, pH 4 and pH 5. Figures 45 and 46 showed the effect of pH of chitosan solution on drug release from the microcapsules prepared from the same 3 hours hardening time with 0.25 gm and 0.50 gm glutaraldehyde content respectively. While Figure 47 showed the same effect on the drug release from the microcapsules prepared form the 1 hours hardening time with 1.0 gm glutaraldehyde content.

From Figures 45-47 the drug release from microcapsules prepared with solution of pH 3 was found to be the highest, follow by that of pH 5, while that of pH 4 showed the lowest. Furthermore it was observed that the microcapsule from solution of pH 5 exhibited more drug release than others in the initial 3 hours then declined to the same level as microcapsules from solution of pH 3 and pH 4. In the cases of 3 hours hardening time as can be seen in Figures 45 and 46, the later 18 hours of the observation shown that the release from solution of pH 5 was closed to that of pH 4. While in the case of 1 hour hardening time it was closed to microcapsule from solution of pH 3, Figure 47.

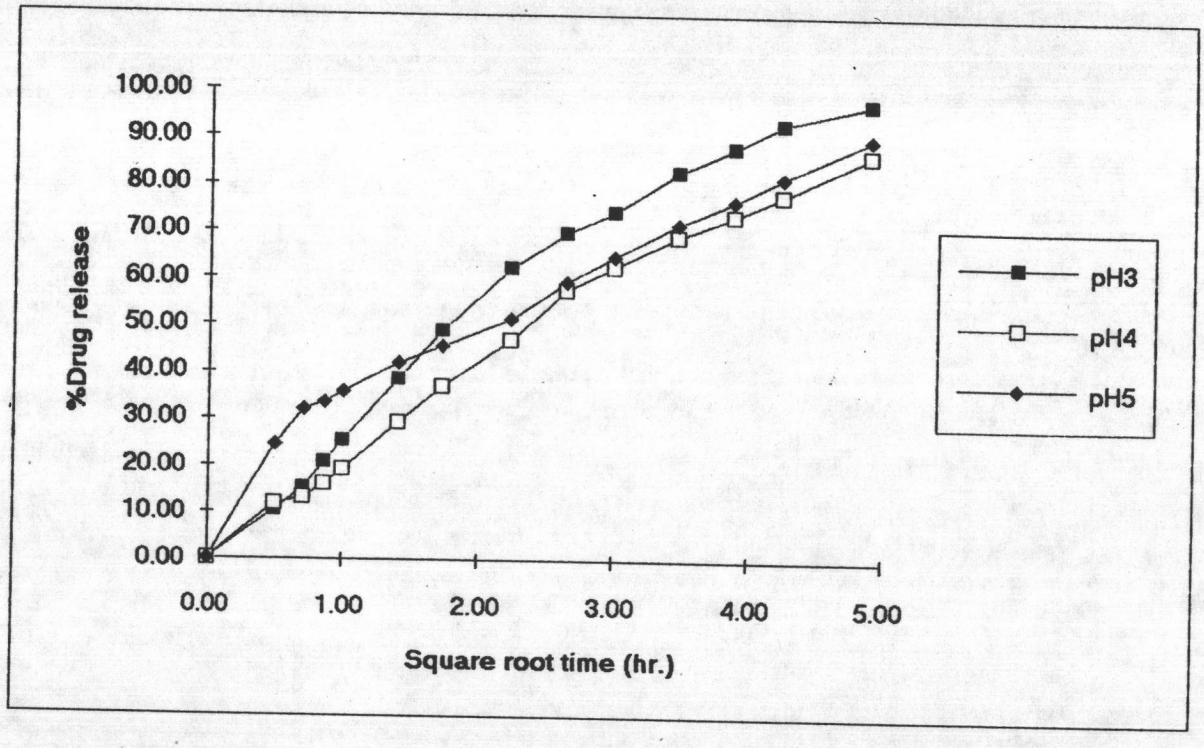


Figure 45 : Effect of chitosan solution pH on Higuchi's plot of indomethacin microcapsules prepared from glutaraldehyde 0.25 gm, hardening time 3 hr

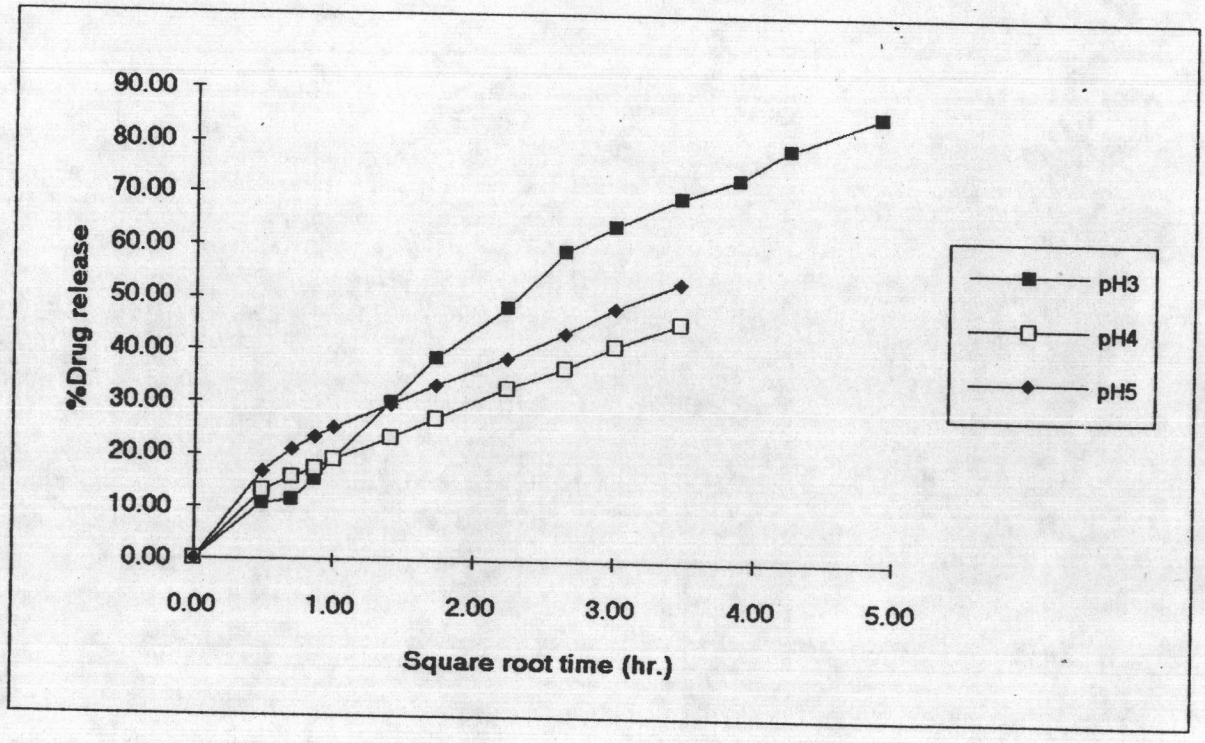


Figure 46 : Effect of chitosan solution pH on Higuchi's plot of indomethacin microcapsules prepared from glutaraldehyde 0.5 gm, hardening time 3 hr

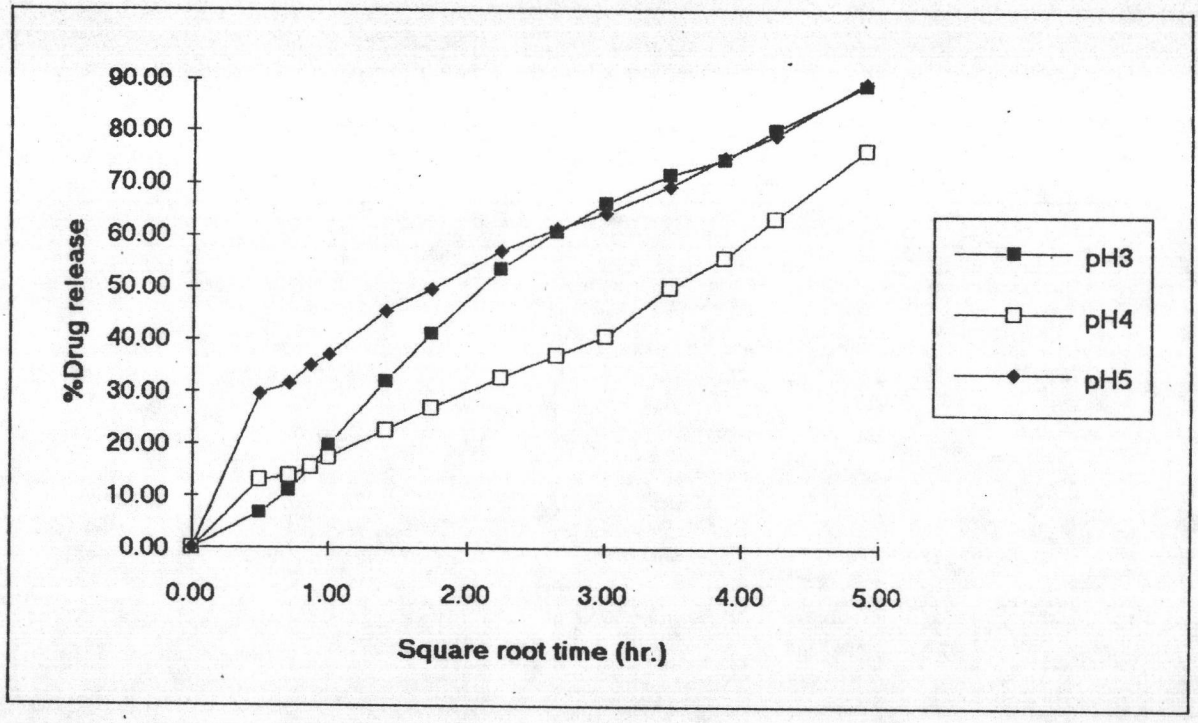


Figure 47 : Effect of chitosan solution pH on Higuchi's plot of indomethacin microcapsules prepared from glutaraldehyde 1.0 gm, hardening time 1 hr

2.4.2 Hardening Time

The microcapsules prepared with various hardening time were observed for their release characteristic. Figure 48 illustrated the Higuchi's plot of microcapsule prepared from chitosan solution of pH 3 and 1.0 gm glutaraldehyde. As expected, the drug release from microcapsules with 1 hour hardening time was higher than those with 3 and 5 hour hardening time. Figures 49 and 50 illustrated Higuchi's plot of microcapsule prepared from 0.50 gm glutaraldehyde with chitosan solution of pH 4 and pH 5 respectively. These results were in contrary to the result obtained from microcapsules prepared with pH 3 of solution, in which 1 hour hardening time gave lower drug release from microcapsule than 3 hour hardening time.

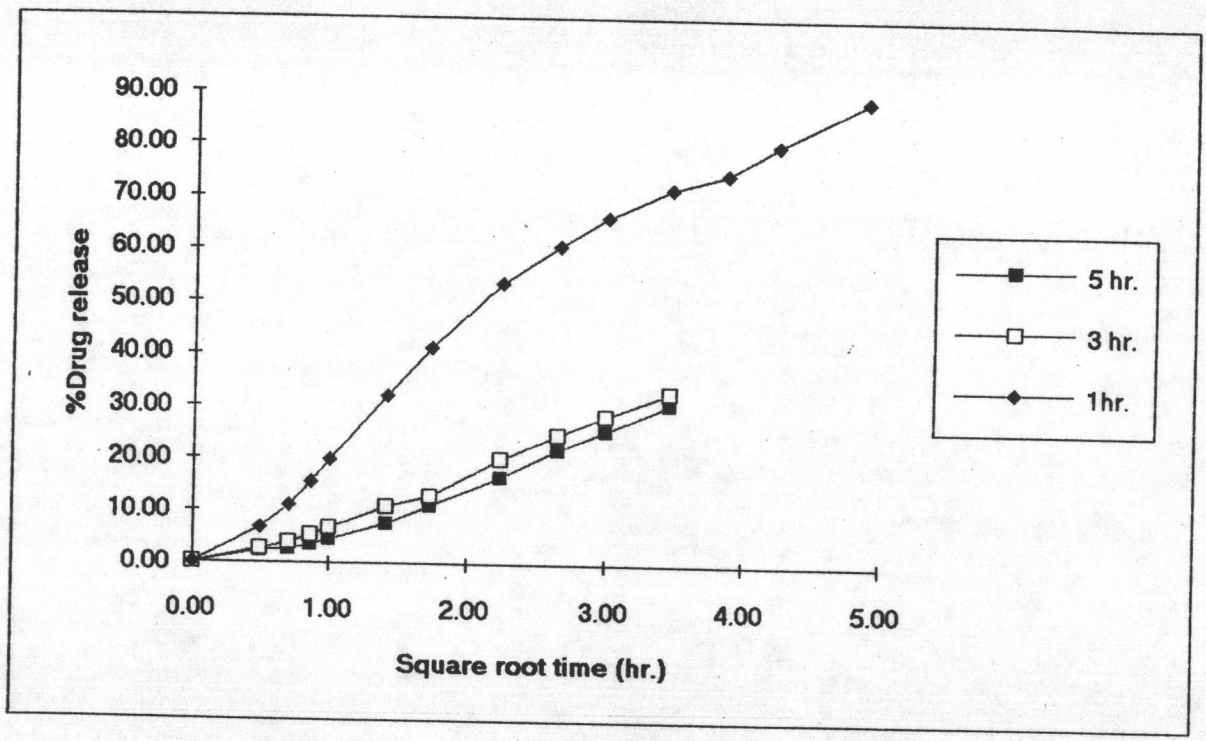


Figure 48 : Effect of hardening time on Higuchi's plot of indomethacin microcapsule prepared from glutaraldehyde 1.0gm, chitosan solution pH3

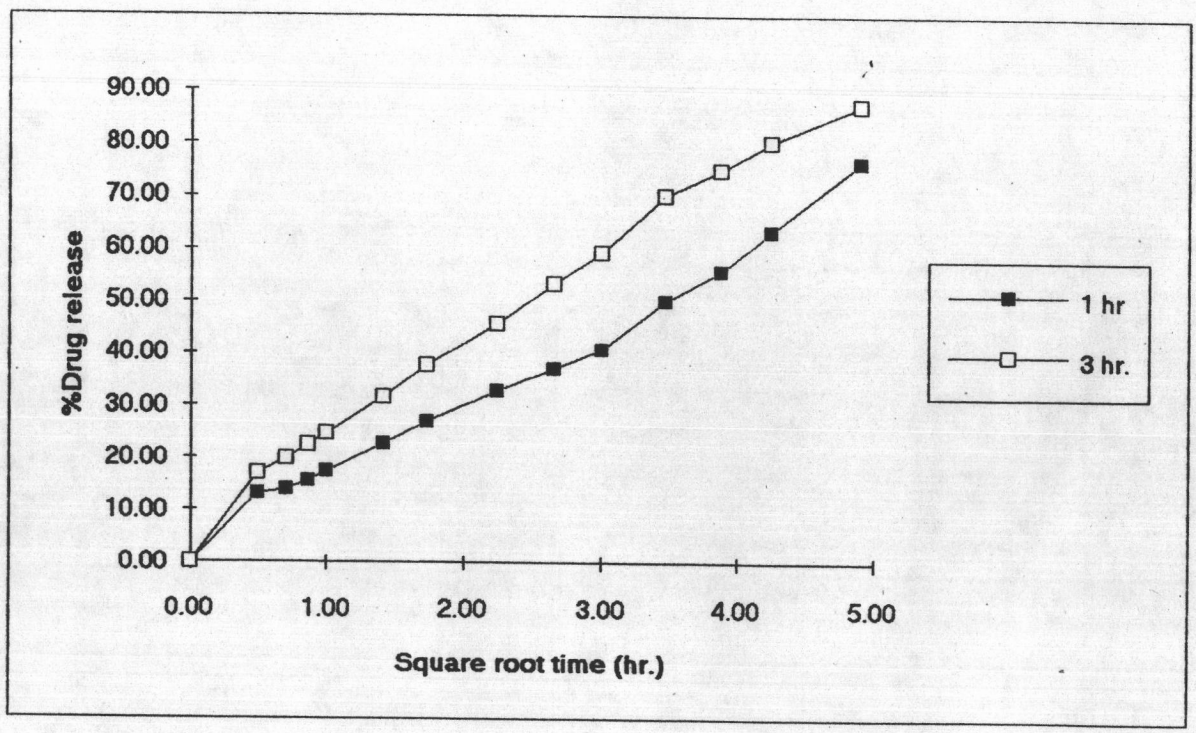


Figure 49 : Effect of hardening time on Higuchi's plot of indomethacin microcapsule prepared from glutaraldehyde 0.5gm, chitosan solution pH4

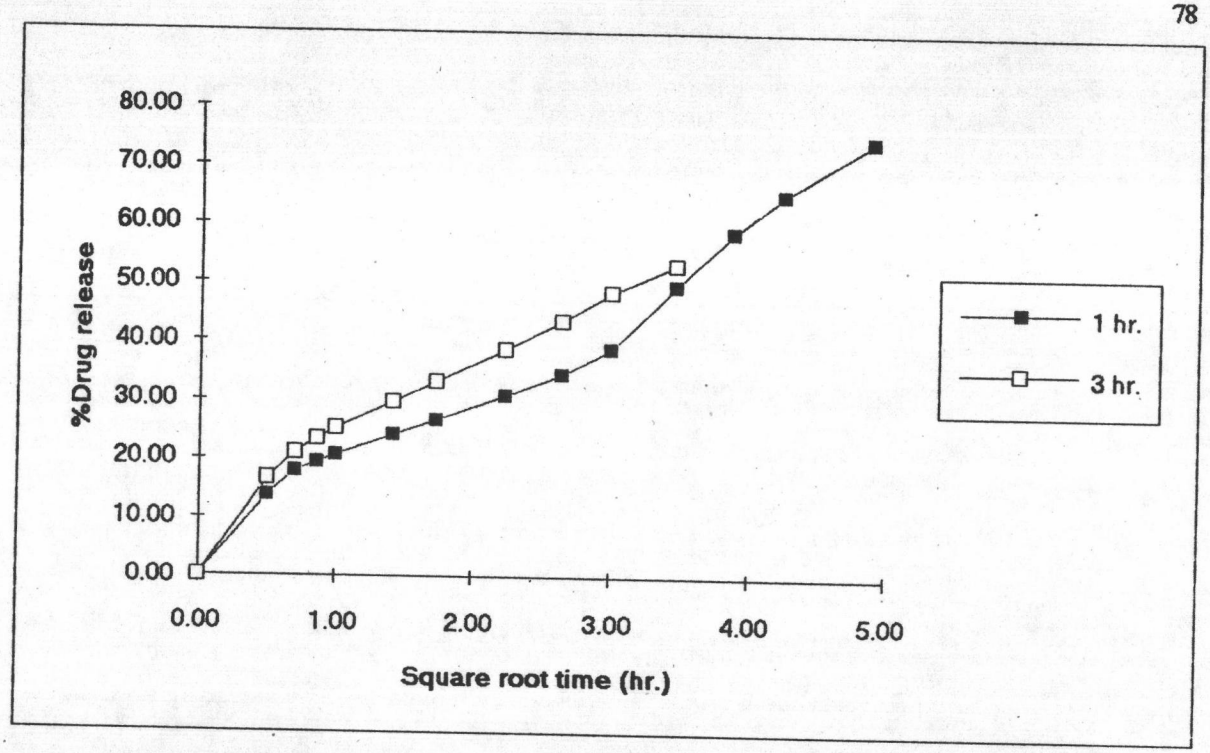


Figure 50 : Effect of hardening time on Higuchi's plot of indomethacin microcapsule prepared from glutaraldehyde 0.5 gm, chitosan solution pH 5

2.4.3 Glutaraldehyde Content

In order to investigated the effect of glutaraldehyde, the percentage drug release of indomethacin microcapsule prepared with different glutaraldehyde content were compared and were shown graphically in Figures 51-56. Figures 51-53 showed the cumulative percent drug release of microcapsule prepared from 3 hours hardening time with chitosan solution of pH 3, pH 4 and pH 5 respectively. While Figure 54 showed the Higuchi's plot of microcapsule prepared from 5 hours hardening time with chitosan solution of pH 3. And Figures 55-56 showed the Higuchi's plot of microcapsule prepared from 1 hour hardening time with chitosan solution of pH 4 and pH 5 respectively. The results shown by these Figures indicated that the amount of drug release decreased when the glutaraldehyde content was increased. But when the glutaraldehyde content was increased to a certain level, there appeared to be a sharp drop in Higuchi's plot to a minimum, then the drug release started to increased again with increased glutaraldehyde content.

Figures 51 and 54 showed the Higuchi's plot of microcapsule prepared from pH 3 of chitosan solution with 3 and 5 hours hardening time respectively. Both of them showed least drug release when 1.0 gm glutaraldehyde was used, it could be seen that the drug release from microcapsules of 1.5 gm glutaraldehyde was closed to that of microcapsules of 1.0 gm glutaraldehyde. At higher glutaraldehyde content of 1.0, 1.5, and 2.0 gm, they showed retard release behaviour in which less than 40% of drug were released within 12 hours. While at lower glutaraldehyde content of 0.25 and 0.50 gm, they showed higher drug release of more than 60 % within 12 hours, which conformed to the USP specification (Table 6).

Figures 52 and 55 illustrated percentage drug release of indomethacin microcapsule from pH 4 of chitosan solution with 3 and 1 hours hardening time respectively. Both of them showed least drug release when 0.50 gm glutaraldehyde was used, the drug release were less than 50%. At 3 hours hardening time, Figure 52, the drug release from microcapsules of 0.25, 1.0, and 1.5 gm glutaraldehyde were not different for the last 18 hours. While at 1 hour hardening time, Figure 55, the drug release from microcapsules of 1.0 gm glutaraldehyde was closed to microcapsules of 0.5 gm glutaraldehyde .

Figures 53 and 56 showed the Higuchi's plot of microcapsule prepared from pH 5 of chitosan solution with 3 and 1 hours hardening time respectively. The results was similar to the microcapsule prepared from pH 4 of solution in which 0.5 gm glutaraldehyde showed least drug release in the both cases, the drug release were lower than 55%.

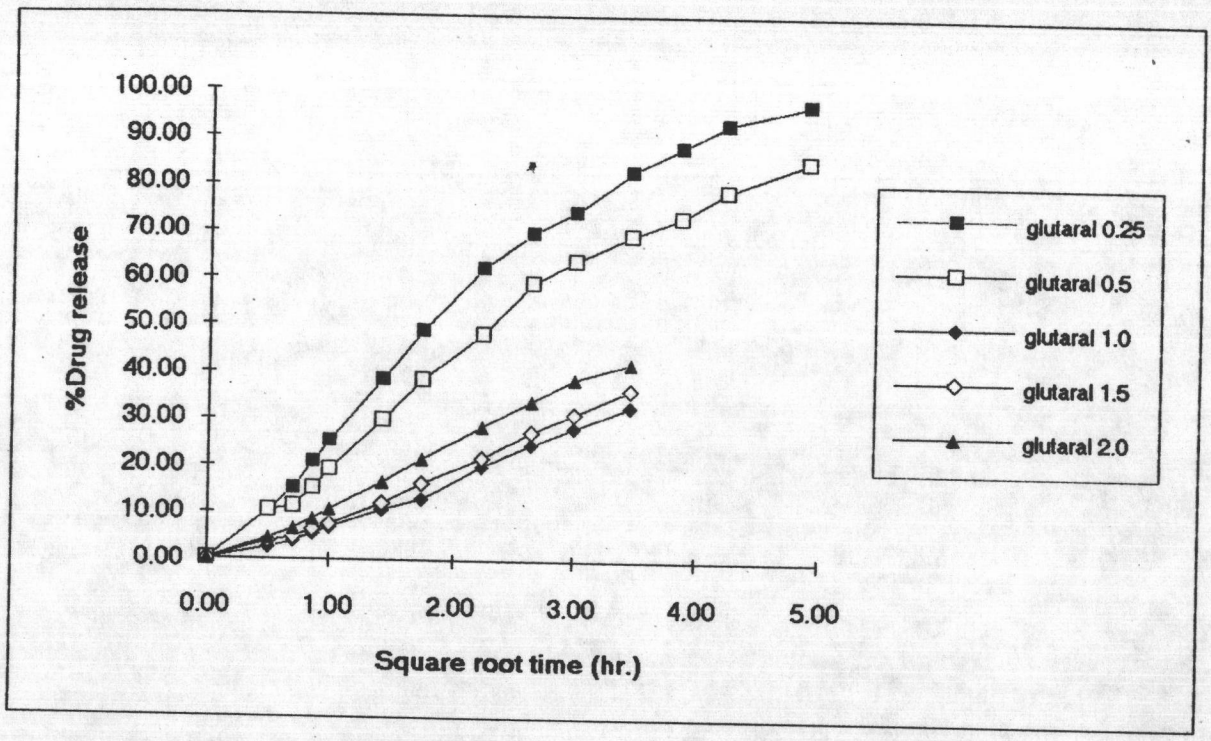


Figure 51 : Effect of glutaraldehyde on Higuchi's plot of indomethacin microcapsule prepared from hardening time 3 hr, chitosan solution pH3

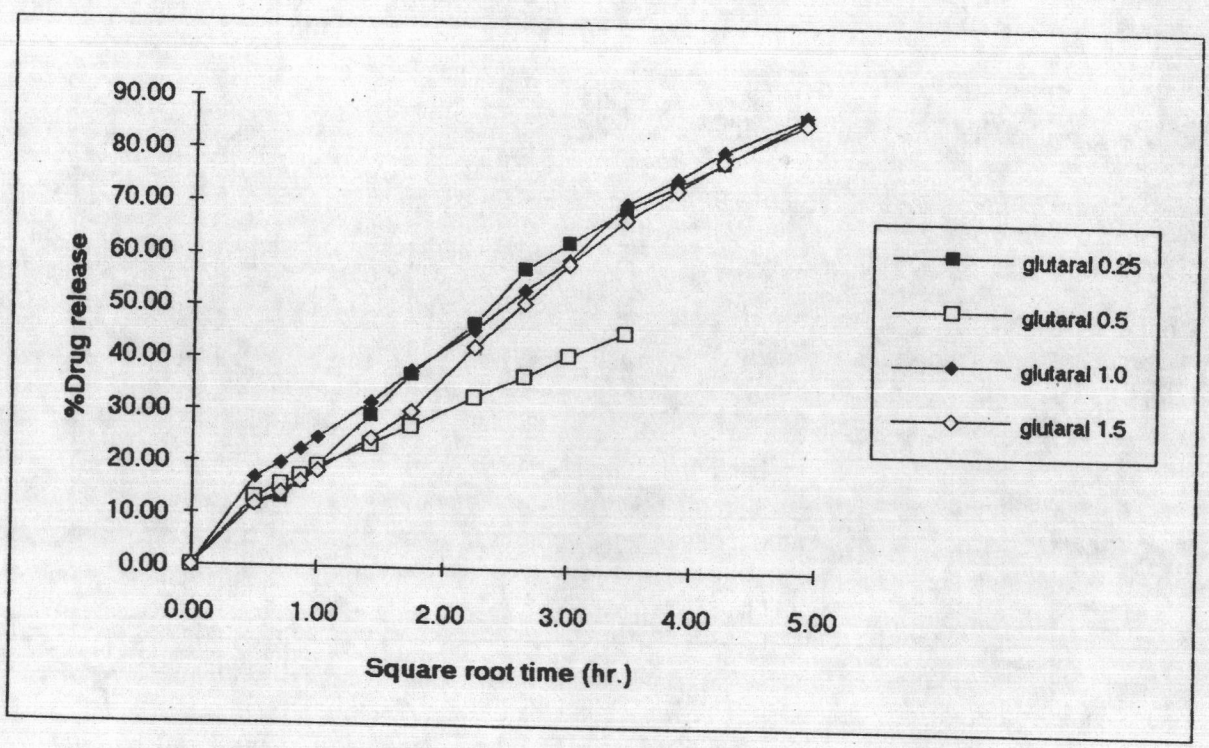


Figure 52 : Effect of glutaraldehyde on Higuchi's plot of indomethacin microcapsule prepared from hardening time 3 hr, chitosan solution pH4

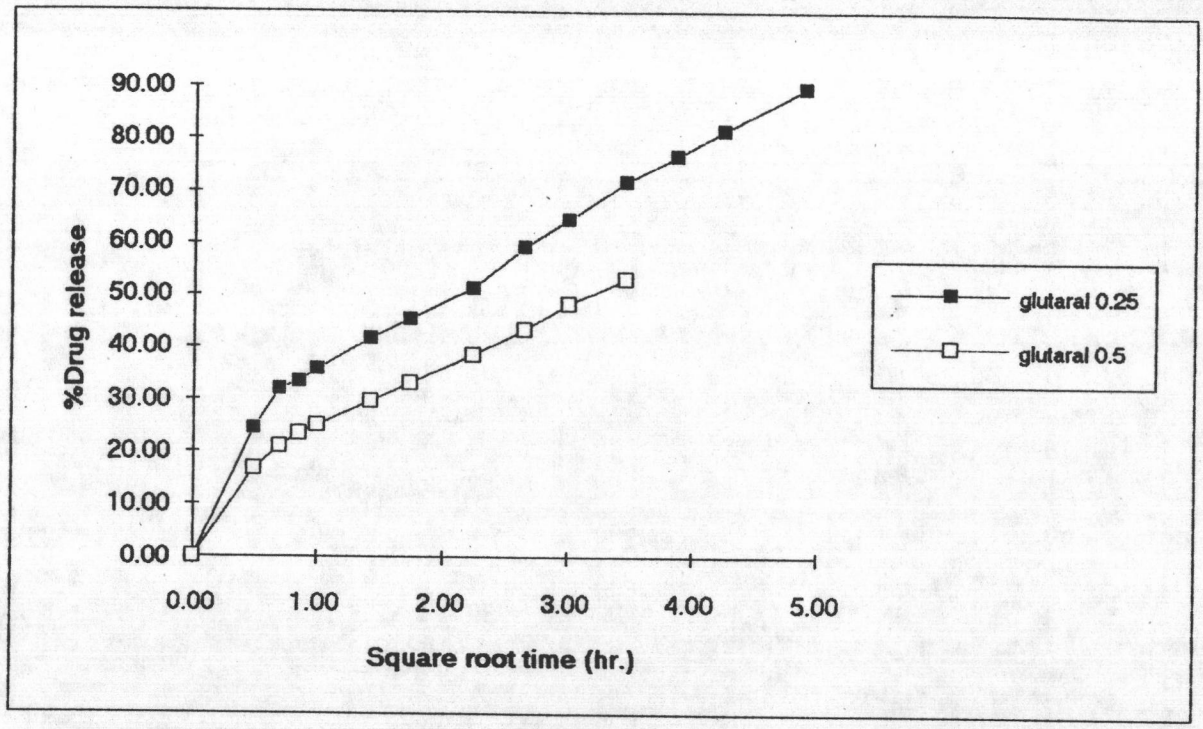


Figure 53 : Effect of glutaraldehyde on Higuchi's plot of indomethacin microcapsule prepared from hardening time 3 hr, chitosan solution pH5

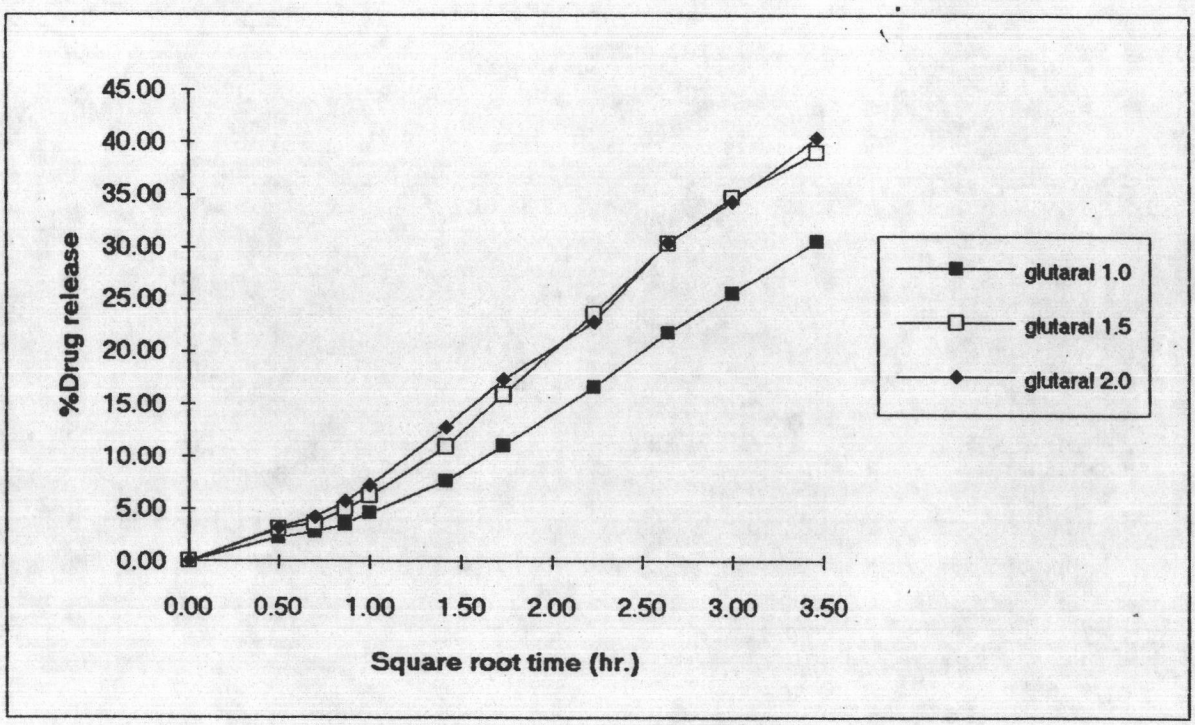


Figure 54 : Effect of glutaraldehyde on Higuchi's plot of indomethacin microcapsule prepared from hardening time 5 hr, chitosan solution pH3

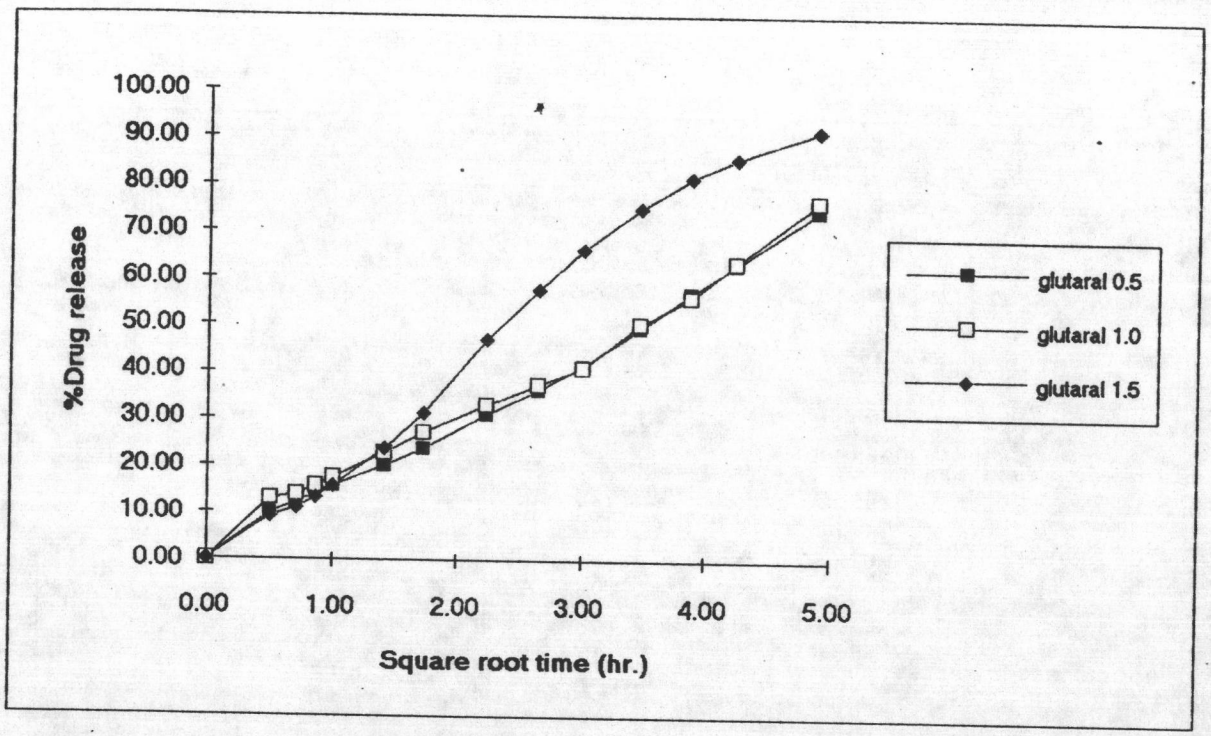


Figure 55 : Effect of glutaraldehyde on Higuchi's plot of indomethacin microcapsule prepared from hardening time 1 hr, chitosan solution pH4

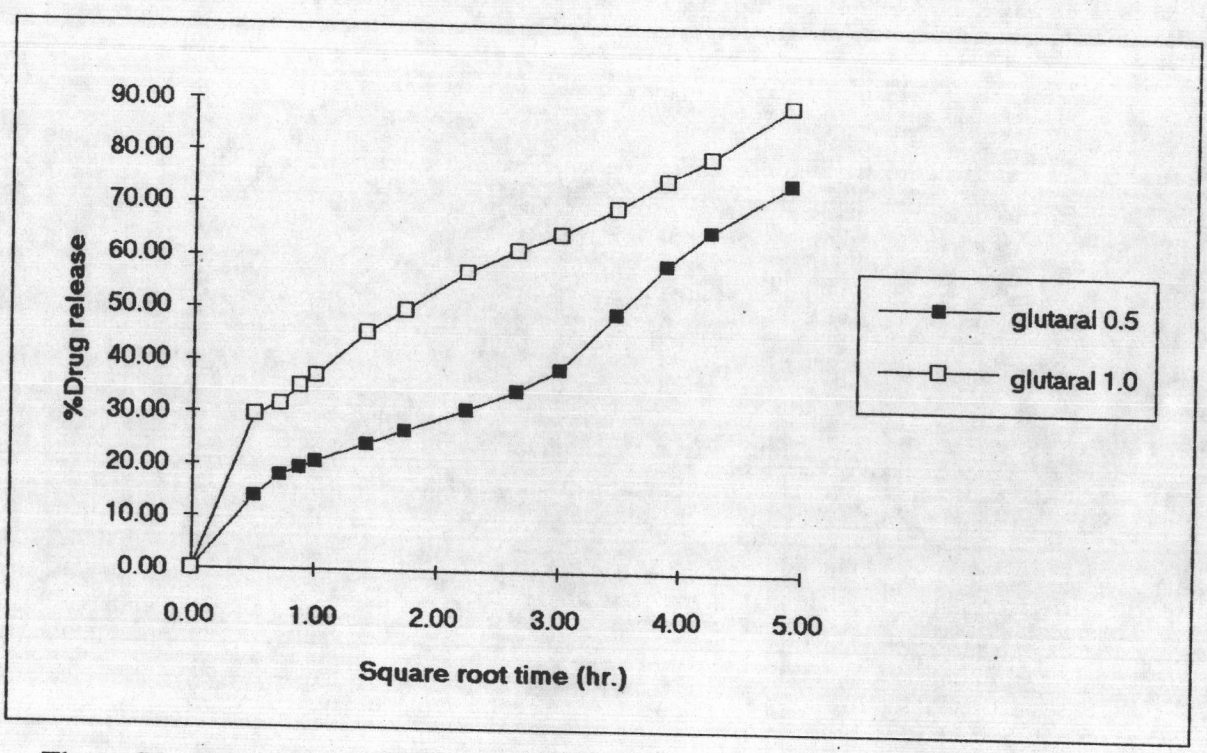


Figure 56 : Effect of glutaraldehyde on Higuchi's plot of indomethacin microcapsule prepared from hardening time 1 hr, chitosan solution pH5

2.4.4 Chitosan Solution Concentration

Figure 57 illustrated the effects of chitosan solution concentration on the Higuchi's plot of microcapsule prepared from pH 4 of chitosan solution, 3 hours hardening time and 0.25 gm glutaraldehyde content. Concentration of chitosan solution was varied in the range of 0.25-1.0% w/v. The drug release from microcapsule prepared with various chitosan solution concentrations were seem to be slight different, which microcapsule of 0.25%w/v of chitosan solution showed more drug release at the first 9 hours of drug release study.

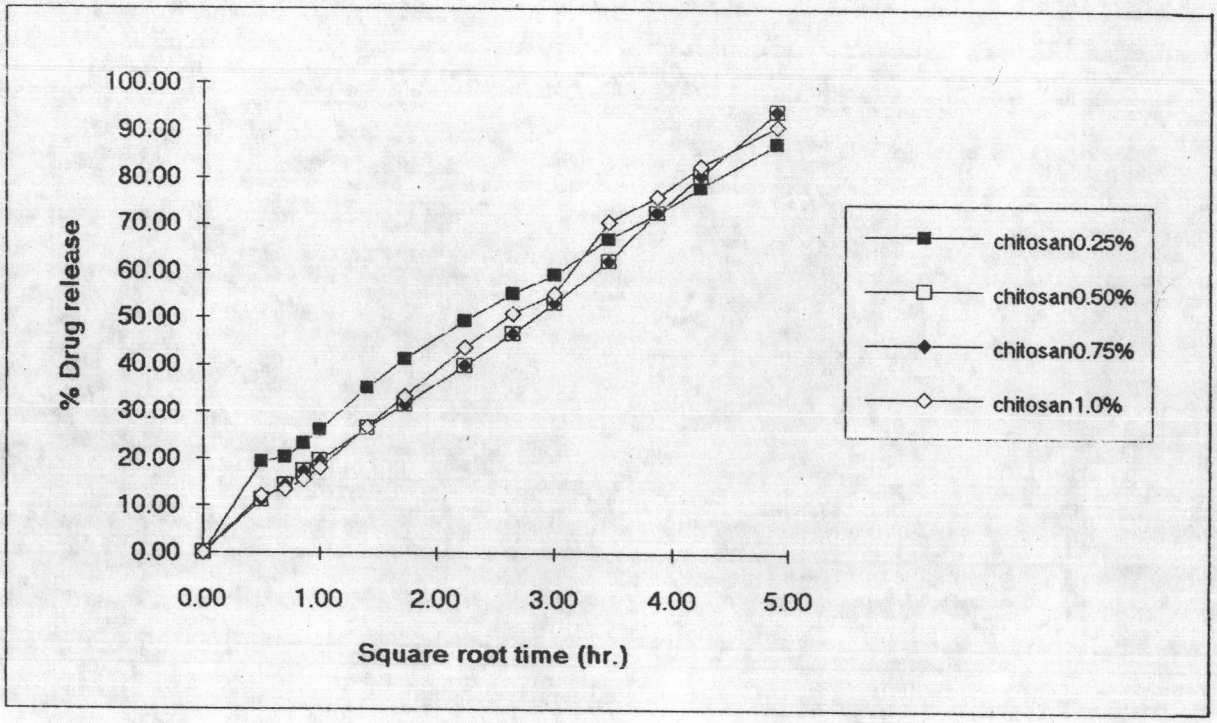


Figure 57 : Effect of chitosan solution concentration on Higuchi's plot of indomethacin microcapsule prepared from chitosan solution pH 4, glutaraldehyde 0.25, hardening time 3 hr

3. Reproducibility Study

Preparation 4 and 16 were chosen for the study of reproducibility of physical properties and drug release pattern of indomethacin microcapsule. The two preparations were prepared from chitosan solution of pH 3 and pH 4 respectively, with hardening time of 3 hour and glutaraldehyde content of 0.25 gm per polymer 1 gm.

3.1 Morphology

Figure 58 showed the scanning electron photomicrographs of batches I-III of preparation 4. All of them showed smooth surface with a little wavy form and they showed some pores on the membrane. The SEM photomicrographs of preparation 16, batches I-III, were illustrated in the Figure 59. From these photomicrographs only slight different in physical characteristics was observed, in which batch II showed some larger size than others. However all of them showed similar creased and heavy wavy form on surface membrane.

3.2 Size and Particle Size Distribution

Figures 60 and 61 showed the frequency curve of size distribution of indomethacin microcapsules prepared from chitosan of pH 3 and pH 4, preparations 4 and 16, respectively. The frequency curve of preparation 4, Figure 60, showed similar pattern which were varied in the range of 47.50-357.50 microns. While frequency curve of preparation 16, Figure 61 showed some different size distribution, where batch II showed larger size than other batches.

The D_{50} value of the indomethacin microcapsule for these two preparations was shown in Table 11. The results showed that the D_{50} value of the three different batches was very similar. Especially in the case of preparation 4, D_{50} value varied in the range of 149.10-151.41 microns. While D_{50} value of preparation 16 varied more in the range of 128.26-147.42 microns.

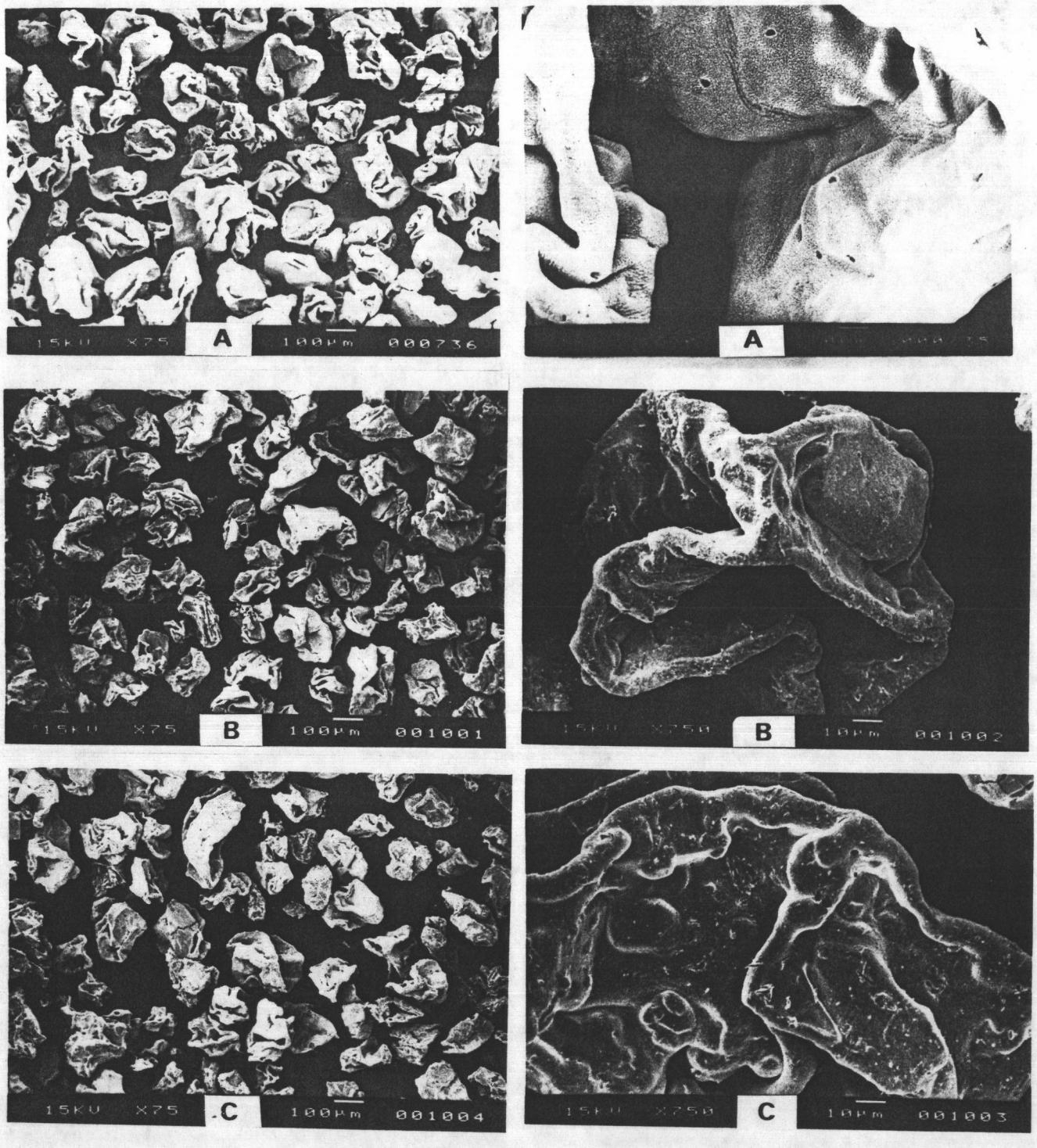


Figure 58 : Scanning electron photomicrograph of indomethacin microcapsule preparation 4, x75 and x750 magnification

- **A : Batch I**
- **B : Batch II**
- **C : Batch III**

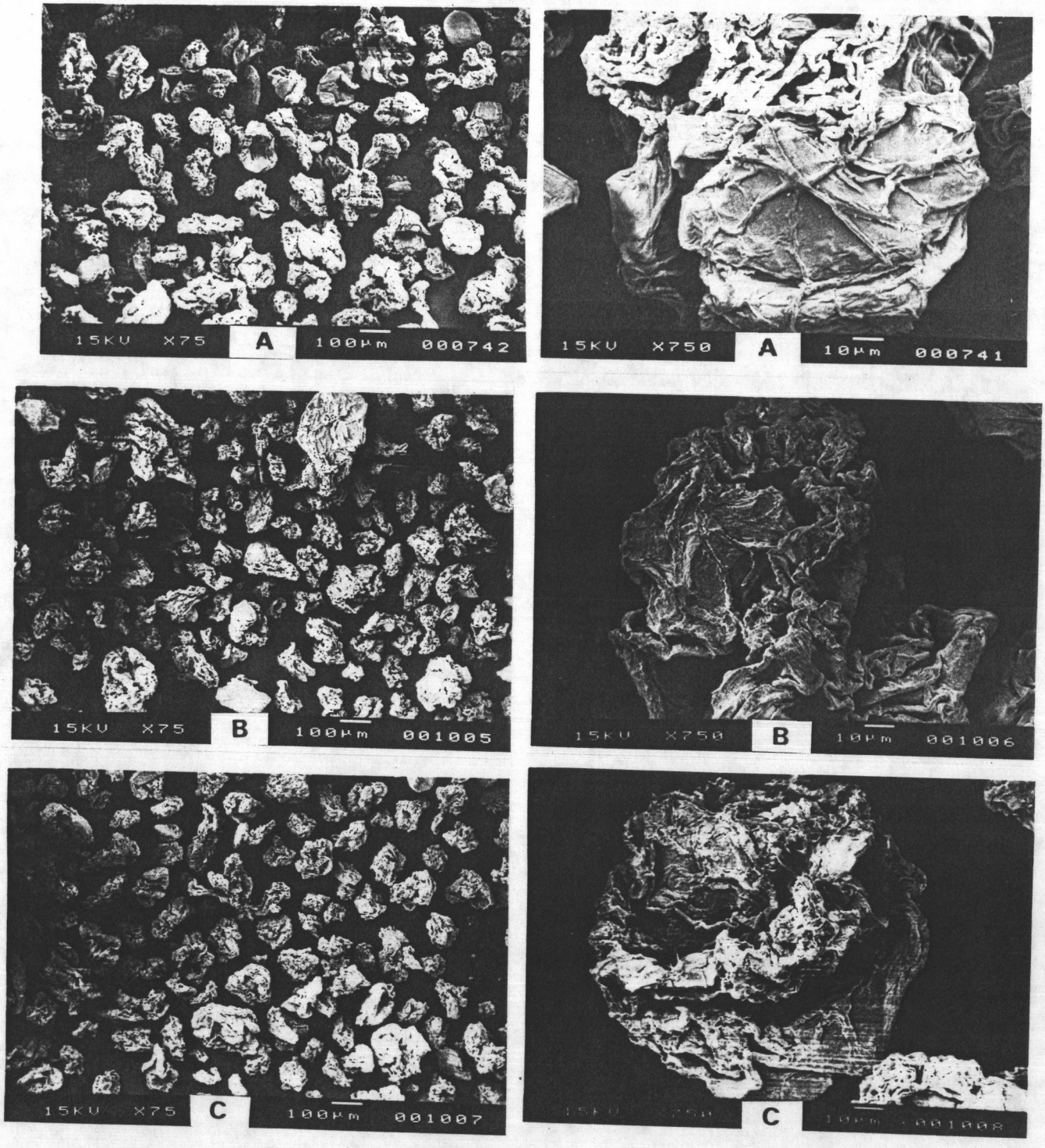


Figure 59 : Scanning electron photomicrograph of indomethacin microcapsule preparation 16, x75 and x750 magnification

- **A : Batch I**
- **B : Batch II**
- **C : Batch III**

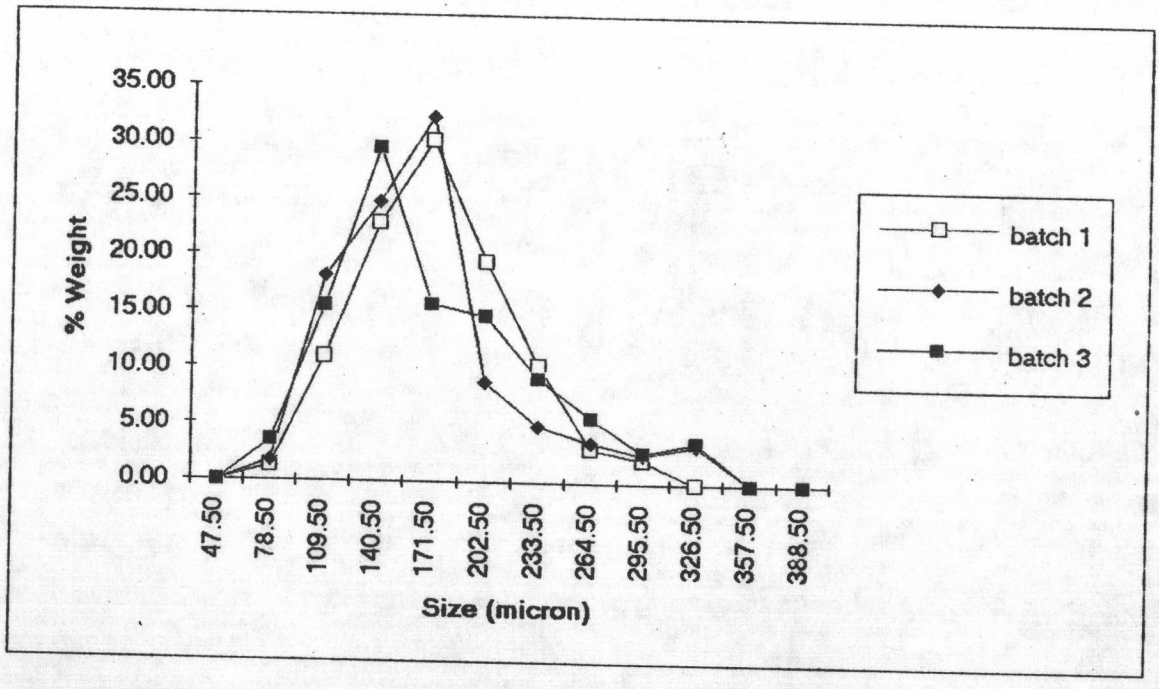


Figure 60 : Frequency curve of various batches, preparation 4 microcapsule.

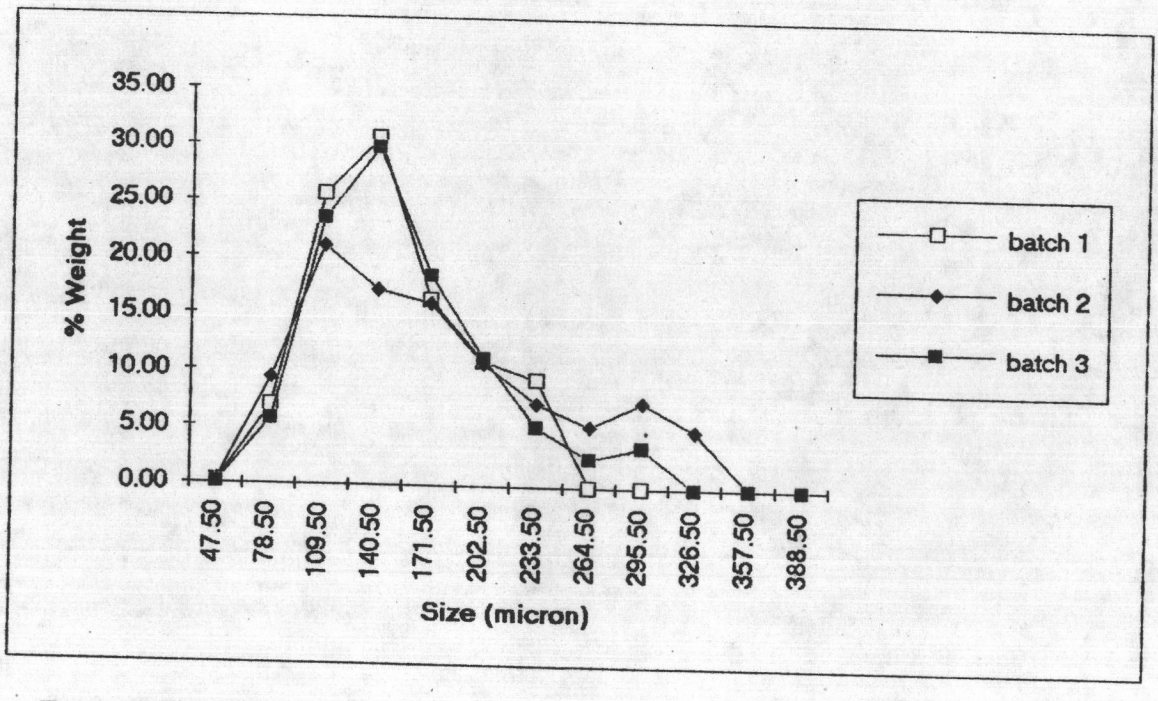


Figure 61 : Frequency curve of various batches, preparation 4 microcapsule.

Table 11 : Geometric mean diameter(D_{50}) of indomethacin microcapsule prepared from various batches

	D_{50} micron)
Preparation 4 :	
Batch I	151.14
Batch II	149.10
Batch III	150.19
Preparation 16 :	
Batch I	128.26
Batch II	147.42
Batch III	134.25

3.3 Drug Entrapment and Drug Recovery

The percentage of drug entrapment and drug recovery of indomethacin microcapsule preparations 4 and 16 were presented in Table 12. It can be observed that preparation 16 showed the narrow range of drug entrapment and drug recovery, which in the range of 41.48-41.89% and 71.35-75.93% respectively. But preparation 4 showed a slightly wider range of drug entrapment and drug recovery, which in the range of 41.94-44.93% and 62.98-77.79% respectively.

Table 12 : Percentage of drug entrapment and drug recovery of indomethacin microcapsule (of various batches)

	% drug entrapment	% drug recovery
Preparation 4 :		
Batch I	42.13	62.98
Batch II	41.94	77.79
Batch III	44.93	71.40
Preparation 16 :		
Batch I	41.89	75.93
Batch II	41.48	71.35
Batch III	41.67	74.93

3.4 Drug Release Study

Figures 61 and 62 showed the Higuchi's plot of microcapsule prepared from chitosan solution of pH 3 and pH 4, preparation 4 and 16, respectively. In both cases there were seem to be no different in Higuchi's plot of three different batches. The Higuchi's plot of each batch in each Figure showed the similar pattern, however batch II showed a slight lower drug release from microcapsules than other batches.

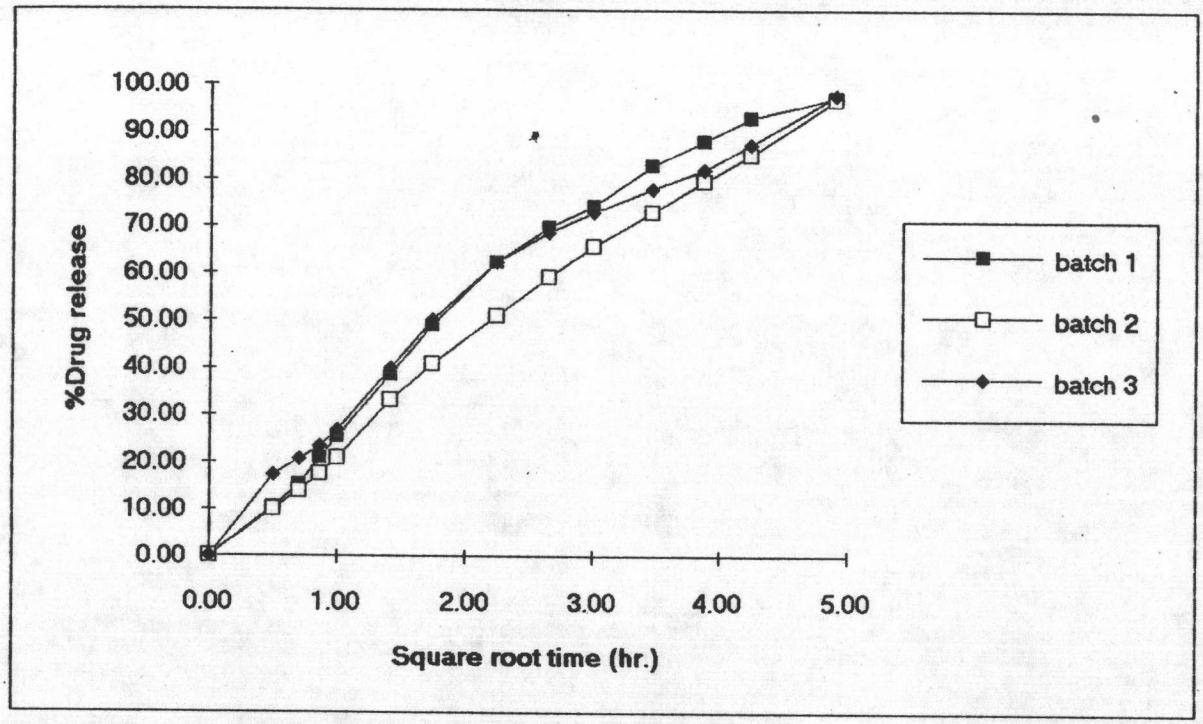


Figure 62 : Higuchi's plot of various batches, preparation 4 microcapsule

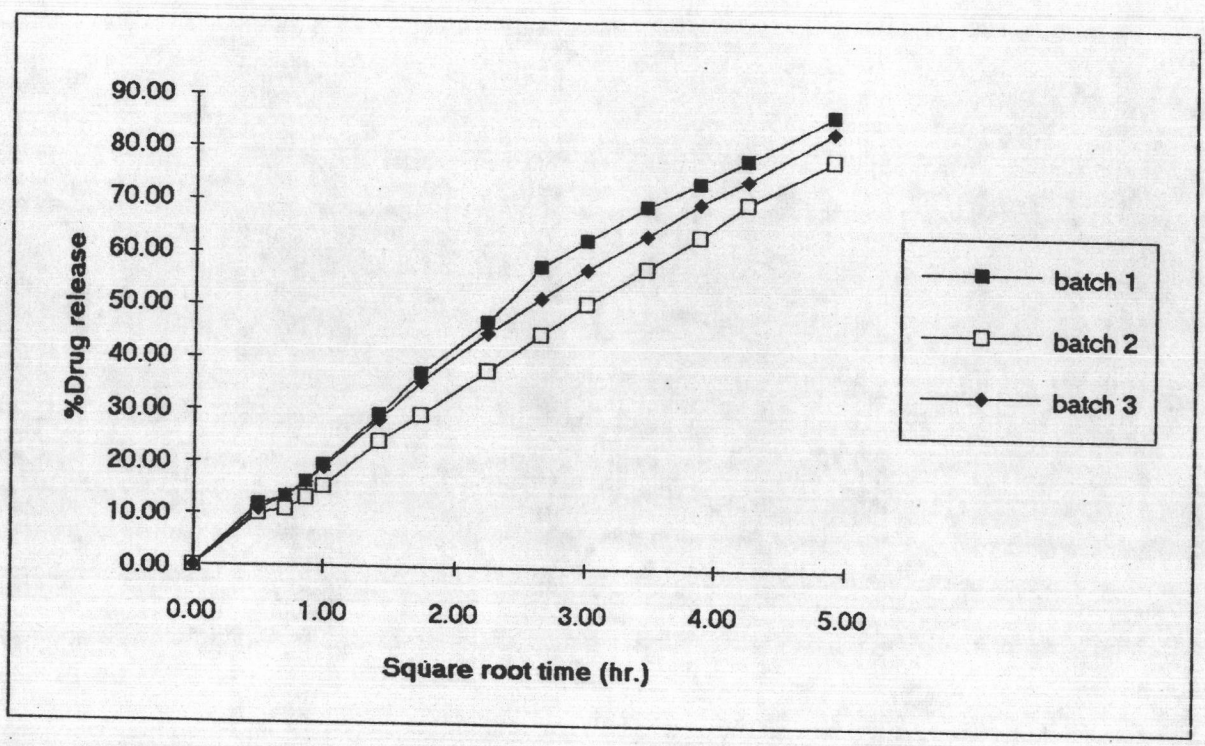


Figure 63 : Higuchi's plot of various batches, preparation 16 microcapsule