Chapter 4

Discussion and Conclusion

In this study, controlled release indomethacin microcapsule were prepared by complex coacervation technique in which drug were entrapped in an aqueous environment. This technique is better than other encapsulation process that used organic solvent which can cause polluted air and is toxic to human.

Most peroral controlled release products are formulated as either encapsulation or tablet. The encapsulated controlled release dosage have two specific advantages over the core tablet designs [Lordi, 1986]. The first is that undisintegrated tablets may remain in the stomach for extended period of time which cause excessive delaying absorption of the maintenance dose. The other advantage is that there is statistical assurance of drug release with encapsulated form, since release of drug by a significant fraction of the granule is highly probable. While if a core tablet fails to release drug all of the maintenance dose is lost.

1. Preliminary Study

Microcapsule prepared from chitosan and CMC solution by complex coacervation technique can be achieved by spraying the CMC solution through a nozzle into chitosan solution method. The microcapsule can be easily prepared without any sophisticate equipment and required only mild conditions for the preparation. The yielded microcapsules had irregular multinuclear structure. The main mechanism for forming the membrane is the electrostatic interaction between positive charged amine group on chitosan chain and the negative charged hydroxyl group on the CMC chain. When the two polymer solutions came into contact the bi-polymer membrane instantaneously formed. The microcapsule looked transparent immediately after the encapsulation process, however it became whitened with time.

Results of the study suggested that the formation of microcapsule could be dictated by varying the solution conditions such as chitosan solution concentration, CMC solution concentration, pH of chitosan solution, and temperature of the process. The effective range for chitosan solution concentration for the preparation of the microcapsule was between 0.25-1.0% w/v while the range for the CMC solution was 1.0-2.0% w/v. The suitable pH for the chitosan solution was pH 3, pH 4 and pH 5, while the processing temperature should not exceed 15°C.

With 0.5% w/v of CMC solution the microencapsulation process could not proceed, this may be because the amount 0.5% w/v CMC solution would cause lower polyion charged density in the system, which was not sufficient for the complex coacervation to occur. The above reasoning was supported by the Voorn-Overbeek theory, in which the lower value of the polyion charge density and molecular weight of the polymer could suppress complex coacervation [Burgess, Kwok and Megremis, 1991].

When the encapsulation process was carried out at 25°C temperature the resulting microcapsule formed groups of chain like structure. It was suspected that the higher processing temperature created high interfacial energy which caused the microcapsules to coalesce in an attempt to reduce the total interfacial energy of the system [Martin, 1993].

In addition to the above solution the physical conditions such as the stirrer speed, spraying pressure of the nozzle and the feed rate of peristaltic pump could also affected the microcapsule formation. It was observed that microcapsule failed to form when using low stirrer speed, or the high spraying pressure or fast feeding rate. Under these conditions the yielded microcapsule were agglomerate and formed chain.

Glutaraldehyde was used as cross-linking agent as it was well known that aldehyde reacted with hydroxyl in CMC chain to form acetal, and it reacted with amino group in chitosan chain to form a schiff base.

The resulting acetal and schiff cross-linked to make the membrane more dense and rigid, and resulting in a net-like surface of the microcapsule. However the intermolecular and intramolecular cross-linking caused both by acetalisation and the formation of schiff base could lessen the capability of hydrogen bonding with water molecule [Kim et al, 1992].

In the recovery process, after washing, nitrogen gas was then used to dry the microcapsule. The advantages of using nitrogen gas for drying over drying at room temperature was that the resulting microcapsules would have more moisture resistance. This was due to the fact that the nitrogen gas when applied would remove the air bubbles on the surface of the microcapsule and would separate the microcapsules preventing them from forming aggregate.

2. Pharmaceutical Study

From the studies of morphology and drug entrapment of pindolol microcapsule, it could be concluded that pindolol, which was a weak base and had a pka of 9.7, could not be encapsulated with this technique. Since the chitosan solution was an acidic, when pindolol microcapsule was initially formed its wall membrane was weak hence the acid could diffuse through the wall membrane and dissolved drug from the microcapsule into the medium during stirring.

In contrary indomethacin, a weak acid with pka 4.5, could be successfully encapsulated by this method as indomethacin did not dissolve in acidic chitosan solution medium.

Hardening time and glutaraldehyde were two factors which affected the microcapsule wall strength. In this study it was observed that with hardening time of 1 hour, 0.25 gm and 0.5 gm glutaraldehyde content, it resulted in tacky agglomerate microcapsule and took longer time for drying. This was due to the incomplete cross-linking in the membrane and hence the polymer chain could form hydrogen bonding with water. When hardening time was extended to 3 hour complete cross-linking prevented this incidence.

At chitosan solution of pH 5, and high glutaraldehyde content the medium became gelly and hence was not possible to collect the yield. The forming of gelly medium could be explained that chitosan was more soluble in lower pH to form NH_3^+. At higher pH chitosan was less soluble, less NH_3^+, hence the junction point for reacting with CMC to form membrane was reduced, and more chitosan molecule were left remaining in the medium. These remaining chitosan molecules cross-linked with the excess glutaraldehyde and turn the medium into gel like substance.

2.1. Morphology of Indomethacin Microcapsule Before and After Drug Release

From the photographs taken by electron microscope, it was observed that the microcapsules prepared with chitosan solution of pH 3 had thicker membrane than microcapsule prepared from those of pH 4 and pH 5 respectively. It was a well known that chitosan required acid to bring glucosamine unit to its soluble form $R\text{-}NH_3^+$ [Skaugrud, 1989]. At pH 3 chitosan could dissolve completely and the molecule become uncoiled. Thus when CMC molecule came into contact with chitosan molecule they instantaneously had complete electrostatic interaction to form wall membrane. When increasing the pH of chitosan solution to pH 4 and pH 5, the soluble form $R\text{-}NH_3^+$ was reduced thus some coil appeared in the chitosan molecule chain, hence when CMC molecule come into contact with chitosan molecule they had incomplete interaction. This resulted in the composition of the wall membrane being made up by more CMC than chitosan, especially for solution of pH 5. This effect showed that yields from solution of pH 5 had more tendency to absorb moisture resulting microcapsule to become agglomerate. Another effect of having more CMC in the wall membrane was that its weaken the microcapsule wall.

The above observation was supported by the SEM photomicrograph of indomethacin microcapsule prepared from chitosan solution of pH 3, pH 4 and pH 5. They showed that at pH 3 the

microcapsule surface was smoother than those of pH 4 and pH 5 respectively.

When studying the SEM photograph traces of indomethacin were found on the surface membrane of some microcapsules. This may be explained by the fact that the microcapsules were washed with IPA in the recovery process. As indomethacin was soluble in IPA, it could be expected that some indomethacin would dissolve in the IPA washing process and some would attach to the membrane. When IPA had evaporated after the nitrogen drying process trace of indomethacin could be noticed on the wall of microcapsule.

Figures 64-66 showed the surface topography before and after drug release of microcapsule prepared from glutaraldehyde 0.25 gm, 3 hours hardening time with chitosan solution of pH 3, pH 4 and pH 5 respectively. In all cases the before drug release surface view of microcapsule showed compact network surface with fine pores on the membrane. While after drug release the surface view of microcapsule showed loosen network surface with larger pores on the membrane.

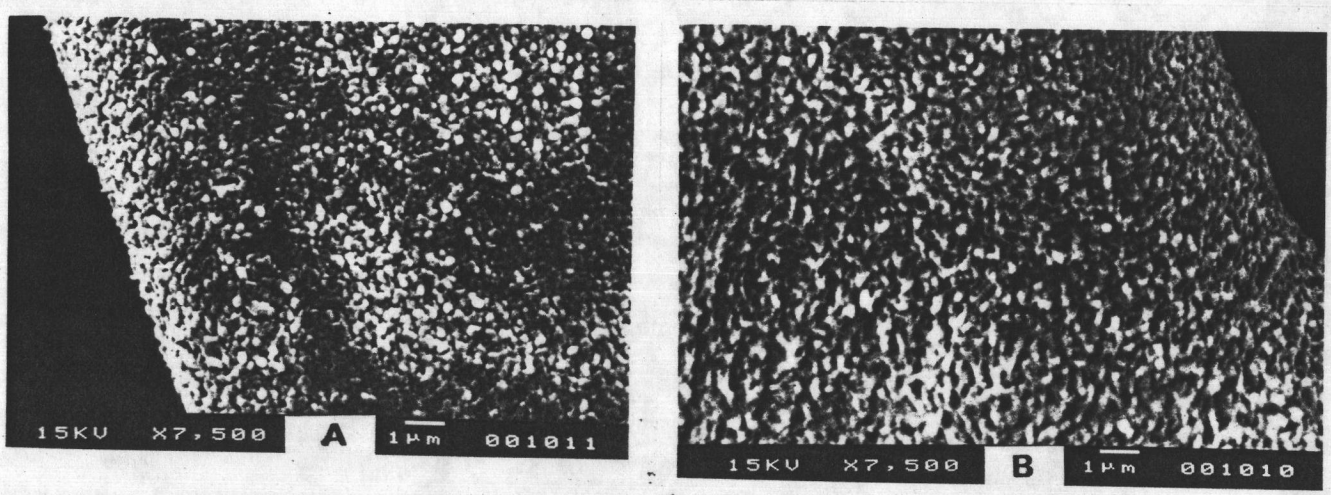

Figure 64 : Scanning electron photomicrograph of indomethacin microcapsule prepared from chitosan solution pH 3, glutaral 0.50 gm, 3 hr hardening time, x7500 magnification

- **A : before drug release**
- **B : after drug release**

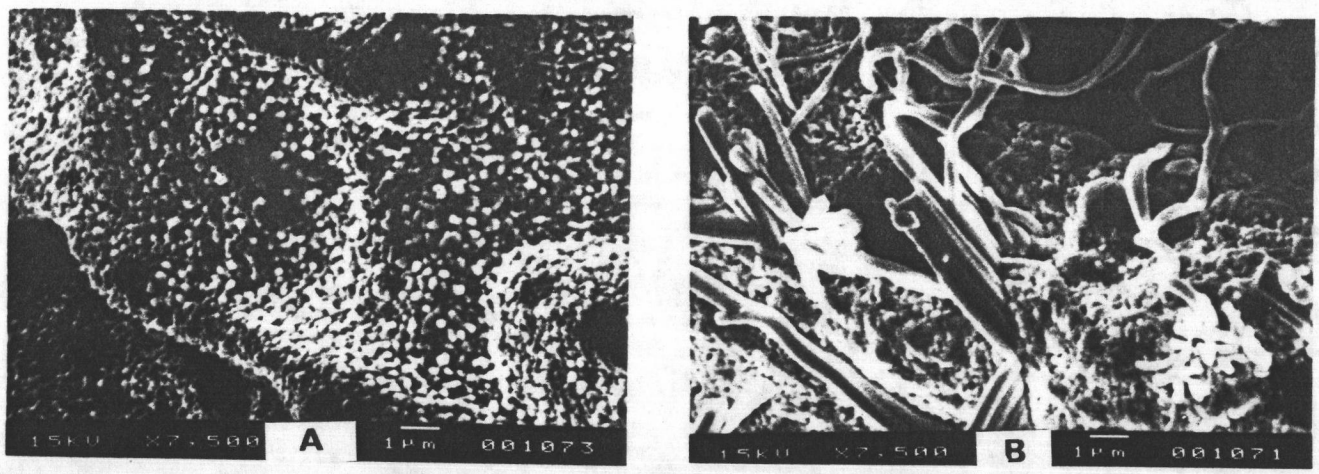

Figure 65 : Scanning electron photomicrograph of indomethacin microcapsule prepared from chitosan solution pH 4, glutaral 0.50 gm, 3 hr hardening time, x7500 magnification

- **A : before drug release**
- **B : after drug release**

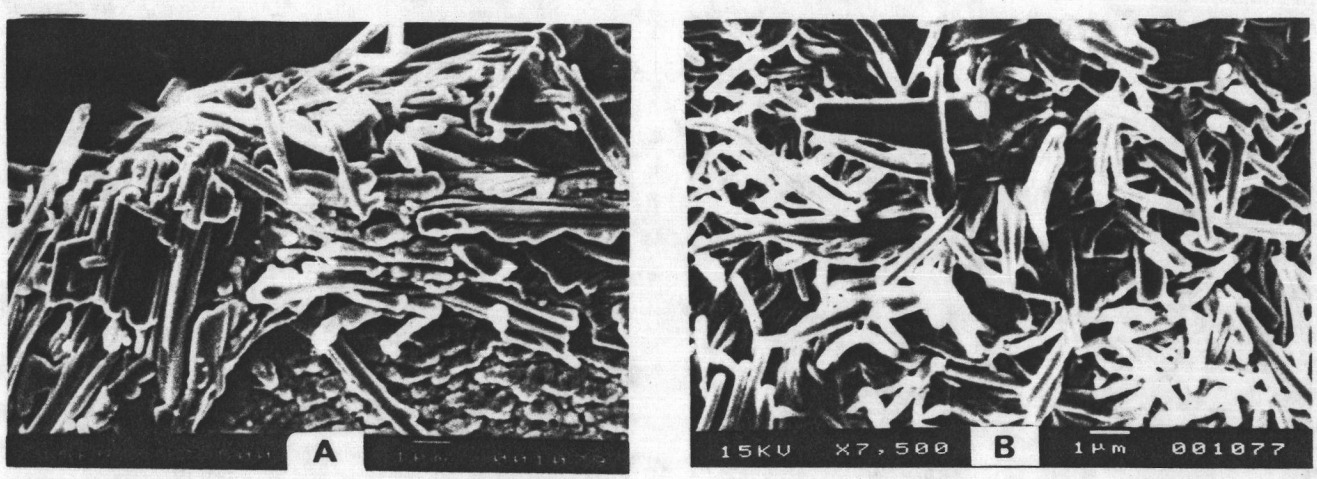

Figure 66 : Scanning electron photomicrograph of indomethacin microcapsule prepared from chitosan solution pH 5, glutaral 0.50 gm, 3 hr hardening time, x7500 magnification

- **A : before drug release**
- **B : after drug release**

2.2 Size and Particle Size Distribution

From the experimental result it was found that processing condition had only slight effect on the particle size distribution of microcapsules. The size distribution varies in the range between 32 - 404 μm, with the majority in the range of 94 - 249 μm.

The solution condition also found to be having little effect on the geometric mean diameter (D_{50}) of the microcapsules. With 1 hour hardening time, the used of 1.0 gm glutaraldehyde content resulted in slightly lower D_{50} value than of 0.5 and 1.5 gm glutaraldehyde content for both cases of microcapsules preparations with chitosan solution of pH 4 and pH 5. This was because the glutaraldehyde caused the membrane to tighten and become tortuous, so when the glutaraldehyde content was increased, the tightening membrane resulting in smaller size. However when the glutaraldehyde content was increased to 1.5 gm, the microcapsule showed slightly increase D_{50} value. This pattern tend to suggest that the amount of glutaraldehyde which caused maximum tightening of the microcapsule membrane was 1.0 gm. This was evident in the preparation with chitosan solution of pH 4 and pH 5. There was no obvious explanation for this result. However other investigator had shown that hardening with formaldehyde produced slightly larger microcapsule and a wider size distribution because the cross-linked membrane produced more strengthen wall which did not reduce in thickness during the recovery process [Nixon and Hassan, 1980].

With 3 hour hardening time and 0.25 gm glutaraldehyde content the preparations resulted in lowest D_{50} value for all the three chitosan solution pH. The D_{50} value was also found to increase with the increased glutaraldehyde content. This may indicate that with longer hardening time, the lower glutaraldehyde content could cause more complete cross-linked membrane than at lower hardening time. The results showed that 0.25 gm glutaraldehyde content gave maximum tightening of the microcapsule membrane.

In addition to the effect of solution condition, the microcapsule size was observed to be affected by the spraying air pressure. When drug CMC dispersion was sprayed through a nozzle and fell dropwise to the chitosan solution, the microcapsule formed instantaneously as the two polymers came into contact. Because of this the size of microcapsule is very much dictated by the size of drug-CMC dispersion droplet. When spraying at high pressure the dispersion droplets are smaller and hence produce smaller microcapsule. This observation was in agreement with other investigators, [Bodmiere and Ornlaksana, 1989].

2.3 Drug Entrapment and Drug Recovery

The experimental results indicate that the chitosan solution pH, hardening time and glutaraldehyde content has no effect on the drug entrapment of the resulting microcapsules. The percentage drug recovery was found to be influenced by the pH of chitosan solution. Chitosan solution of pH 3 showed greatest value of percentage drug recovery.

The percentage drug recovery varied in the range 50.98-86.67%. This might be due to that indomethacin microcapsule could be lost during the washing process since it was soluble in IPA.

The yield from chitosan solution of pH 3 was washed with IPA only once while others were wash 4 times. Its wall membrane, as discuss earlier, were also stronger than the other two, hence these factors contributed to them having greatest drug recovery value as less drug were loss during washing or through weak membrane.

The concentration of chitosan solution affected the drug entrapment and drug recovery. Higher concentration gave higher percentage of drug entrapment and drug recovery. This was due to the microcapsule wall was more harden with higher concentration of chitosan solution, since the membrane was more chitosan than CMC molecule. Therefore, in the washing process, in the higher

concentration of chitosan solution microcapsule were less indomethacin loss than in the lower concentration of chitosan solution microcapsule.

2.4 Drug Release Study

In the drug release study, linear regression was used to compute the correlation coefficient (r^2) of percentage drug release against time, percentage drug release against square root time and log percentage drug remained against time. These values were then used to find the kinetic pattern of indomethacin microcapsule. Table 13 showed these correlation coefficients of all indomethacin microcapsule formulations. It could be observed that the maximum values in all cases occurred at square root time, hence the kinetic pattern of the indomethacin microcapsule was a square root time kinetic pattern.

The analysis of SEM photomicrograph and the Higuchi's plot which were described by a linear square root time dependence, showed that the kinetic of drug release from microcapsule exhibited a mass transportation phenomenon. The wall membrane of the microcapsule showed matrix properties, such as porosity and tortuosity, that fitted the granular type matrix which cause square root time drug release pattern of the microcapsule. These results were in agreement with many other investigators who had reported that drug release from microcapsule produced by coacervation technique was proportional to the square root time [Deasy, 1989].

The microcapsule drug release mechanism involved the permeation of dissolution medium through the wall membrane then the drug was dissolved and diffused through the pore or membrane which was damaged during the recovery process. In addition the mechanism may involve time erosion of membrane or rupture of the barrier after sufficient moisture has permeated through the membrane. Any or a combination of these mechanisms could be a rate limiting step in the drug release process.

Table 13 : The correlation coefficient of % drug release vs. time, % drug release vs. square root time, and log % drug remain vs. time

Preparation	correlation coefficient		
	% drug release		log % drug remained
	vs. time	vs. square root time	vs. time
1	0.9916	0.9873	0.8917
2	0.9823	0.9898	0.8613
3	0.9834	0.9935	0.8433
4	0.9759	0.9940	0.7441
5	0.9696	0.9963	0.8556
6	0.9822	0.9947	0.8459
7	0.9803	0.9965	0.8258
8	0.9630	0.9972	0.7835
11	0.9751	0.9920	0.7618
13	0.9742	0.9967	0.7184
14	0.9592	0.9946	0.6743
15	0.9888	0.9886	0.7649
16	0.9694	0.9977	0.7075
17	0.9227	0.9874	0.6222
18	0.9365	0.9929	0.6219
19	0.9948	0.9953	0.9581
21	0.9507	0.9760	0.6341
22	0.8050	0.9376	0.5161
24	0.8257	0.9408	0.5132
25	0.8997	0.9753	0.5754
28	0.9127	0.9872	0.5986
29	0.9457	0.9984	0.6660
30	0.9642	0.9976	0.6952

	correlation coefficient		
	% drug release		log % drug remained
	vs. time	vs. square root time	vs. time
Preparation 4			
batch I	0.9758	0.9940	0.7441
batch II	0.9581	0.9979	0.7069
batch III	0.9513	0.9957	0.6747
Preparation 16			
batch I	0.9694	0.9977	0.7075
batch II	0.9643	0.9988	0.7275
batch III	0.9577	0.9989	0.6954

Tables 25 and 26 contained drug release data of different preparations of indomethacin microcapsule. All preparations in Table 25 have drug release conforming to the USP specification in Table 6. While Table 26 showed those preparation which failed to conform. All processing conditions had effects on the drug release profile except the concentration of chitosan solution, which would be further discussed.

2.4.1 Chitosan solution pH

From the drug release study it could be seen that microcapsule prepared from chitosan solution of pH 3 had fastest drug release. With pH 4 and pH 5 their Higuchi's plots were very similar, the notable exception was in the initial stage where those from solution of pH 5 showed the fastest drug release. It was believed that those prepared from solution of pH 4 and pH 5 has slower drug release than that of pH 3 because the latter had the smoothest surface and more porous while the former had high tortuousity membrane with rough and creased surface. A high tortuousity meant that the effective average diffusion path was large hence the reduction of drug release capability of the microcapsule. The porosity helped to increase the avenues for drug release hence faster release from microcapsule.

The scattered indomethacin on the surface microcapsule of pH 5 was believed to cause the fast initial release rate in microcapsule of pH 5. The microcapsule of pH 5 when observed with electron microscope were found to have some rupture membrane. As previously discussed this was because it absorbed more moisture than the other and caused damage to the microcapsule surface when washed with water and later with IPA, hence the excess indomethacin on the outside surface.

2.4.2 Hardening time

For microcapsule prepared from chitosan solution of pH 3 with longer hardening time of 3 hour the drug release from microcapsule was slower than that of microcapsule with shorter hardening time of 1 hour. The reason for this was evident from that longer hardening time allowed the cross-linking process to be more complete and strengthening the microcapsule wall hence slower drug release from microcapsule.

In contrary for microcapsule prepared from chitosan solution of pH 4 and pH 5 with longer hardening time of 3 hour the drug release from microcapsule were faster than that of microcapsule with 1 hour hardening time. There is no explanation for these results, further study was to investigate.

2.4.3 Glutaraldehyde solution content

The drug release of microcapsule generally decreased with the increase in glutaraldehyde content until a point was reached where a sharp drop to the minimum release rate occurred then the rate increased again with the increasing content. The content in which rate drop sharply in the experiment was 1.0 gm per polymer 1 gm with chitosan solution of pH 3, hardening time 3 hour, and 0.5 gm per polymer 1 gm with chitosan solution of pH 4 and pH 5, hardening time 1 and 3 hour.

The initial dropped in drug release when increasing the glutaraldehyde content was because the increased amount of glutaraldehyde help strengthen and increased the tortuousity of the microcapsule wall membrane in the cross-linking process. After the minimum drug release, increased glutaraldehyde content caused the drug release to increase again. This may be due to the excessive hardening treatment could cause the membrane to become brittle [Nakatsuka, Anthony and Andrady, 1992] and cracking, which was observed on the

1.5 and 2.0 gm glutaraldehyde content microcapsule. The result was in agreement with other investigator [Luzzi and Gerraughtly, 1967].

2.4.4 Concentration of Chitosan Solution

The results from drug release study of indomethacin microcapsule prepared from different concentration of chitosan solution showed that there was no significant different in Higuchi's plot slope of each preparation ($p \geq 0.05$). The Higuchi's plot slopes data were shown in Table 27 in Appendix while Table 14 showed the variance analysis of these Higuchi's plot slope. It indicated that the concentration of chitosan solution did not affected the drug release from microcapsule, which was disagreed with other investigator [Kim and Rha, 1989].

Table 14 : Variance analysis of Higuchi's plot slope at different concentration of chitosan solution microcapsule

Source of Variation

	SS	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	9.626092	3	3.208697	1.425597	0.305251	4.06618
Within Groups	18.0062	8	2.250775			

3. Reproducibility

Reproducibility study was conducted on preparation 4 and 16, with 3 batches of each were investigated. The comparison of the indomethacin microcapsule morphology, size, size distribution and percentage of drug entrapment and drug recovery of these 3 batches showed no significant different and indicated that reproducibility was highly probable for this microencapsulation method. The result of drug release study also showed no significant different in Higuchi's plot slope of various batches ($p \geq 0.05$). The variance analysis of Higuchi's plot slope of preparation 4 and 16 was shown in Table 15 and 16 respectively. The Higuchi's plot slopes data were shown in Table 28 in Appendix.

Table 15 : Variance analysis of Higuchi's plot slope at different batch of formulation 4 microcapsule

Source of Variation

	SS	df	MS	F	P-value	F crit
Between Groups	10.19627	2	5.098133	0.546446	0.605318	5.143249
Within Groups	55.97773	6	9.329622			

Table 16 : Variance analysis of Higuchi's plot slope at different batch of formulation 16 microcapsule

Source of Variation

	SS	df	MS	F	P-value	F crit
Between Groups	0.639356	2	0.319678	0.046209	0.955179	5.143249
Within Groups	41.50887	6	6.918144			

4. Conclusion

Chitosan is a cationic polymer which is soluble in acid and CMC is an anionic polymer which is soluble in water. the two polymer can be used for the preparation of controlled release microcapsule by complex coacervation technique. The encapsulation is based on the electrostatic interaction where both polymer instantaneously form microcapsule when come into contact.

The drug which can be used for the encapsulation should be insoluble in both water and acid. In addition this process is believed to be suitable for microencapsulation of drug in liquid form, or the drug which is heat sensitive, or cell culture because of the mild conditions involved in the process especially the low temperature ($\leq$ 15°C). The essential factors for microcapsule formation involves both the physical and solution conditions. The solution conditions also effect the morphology and drug release kinetic of the yielded microcapsules. These effects are summarised as followed.

- Microcapsules prepared with chitosan solution of pH 3 had the smoothest surface in comparison to those prepared with pH 4 and pH 5.
- Fastest drug release was achieved by the microcapsules prepared with chitosan solution of pH 3.
- Microcapsules prepared with chitosan solution of pH 3 had lower drug release when increased hardening time.
- Microcapsules prepared with chitosan solution of pH 4 and pH 5 had lower drug release when decreased hardening time.
- Microcapsules prepared with glutaraldehyde 1.0 gm per polymer 1 gm had lowest drug release at chitosan solution of pH 3.
- Microcapsules prepared with glutaraldehyde 0.5 gm per polymer 1 gm had lowest drug release at chitosan solution of pH 4 and pH 5.
- The concentration of chitosan solution has no effect on the drug release from the microcapsules.

In conclusion this method of microencapsulation by aqueous complex coacervation should be an attractive mean for pharmaceutical manufacturing as it does not used organic solvent which cause polluted air and is toxic to human. The used of chitosan and CMC polymers for the microencapsulation simplify the preparation process in that the microencapsulation can be done in a relatively mild conditions in comparison with other aqueous polymers such as gelatin and acacia. Furthermore controlled release can be successfully achieved with this combination of chitosan and CMC polymer for the microencapsulation.

This study had shown that physical characteristics and drug release of microcapsule were reproducible by this method of microencapsulation. This reproducibility property is highly desirable for the method be used for industrial scale production of microcapsules. However this is the first time, study has been conducted to investigate the used of chitosan and CMC to prepare controlled release microcapsule by complex coacervation technique. More study should be done to further investigate this technique before it can be used by the industry.