CHAPTER IV

RESULTS AND DISCUSSION

4.1 Preliminary Study

In the preliminary study, the viscosity of the chitosan solution played an important role in hydrogel bead preparation process. Therefore, the variations were investigated as follows :

i. The chitosan to drug ratios
ii. The concentrations of TPP solution
iii. The pH value of TPP coagulant solution
iv. The bead immersion time in the coagulant solution

Next, the size and shape of the obtained beads and drug loading efficiency (%LE) were evaluated. The percentage of loading efficiency in each chitosan bead formulations with or without DS, was given in Table 4.1.

From Table 4.1, formulations A and B are the beads without DS. The ratios of chitosan to DS were varied, referred as from Formulations C to I. The results showed that the %LE was not significantly different. Therefore, the most suitable formulation may be considered from the results of the encapsulation efficiency, given in Table 4.4.

Table 4.1 Drug loading efficiency (LE) of the chitosan/DS beads in the preliminary study of polymer/drug ratio

Formulation	Ratio of the compositions				NaOH conc.	TPP pH 6.0	Time	% [a]LE ± [b]S.D.
	CS	PEG	DS	%GD (v/v)	(% w/v)	(% w/v)	(minute)	
A	1/0	1/0	-	-	5	-	20	-
B	1/0	1/0	-	-	-	1	20	-
C	1/0	3/1	0.33	-	-	1	20	97.59±0.23
D	1/0	2/1	0.50	-	-	1	20	98.09±0.08
E	1/0	1.5/1	0.67	-	-	1	20	98.03±0.14
F	1/0	1/1	1	-	-	1	20	97.54±0.22
G	1/0	1/1.5	1.5	-	-	1	20	97.25±0.18
H	1/0	1/2	2	-	-	1	20	95.34±0.10
I	1/0	1/3	3	-	-	1	20	nd

[a] %LE = The percentage of drug loading efficiency was studied using indirect method. [28]

[b] S.D. = Standard deviation

nd = not determined because the polymer mixture solution was too high viscosity to form beads.

4.2 Characterization and Physical Properties

4.2.1 Size and shape of the beads

In this section, the effects of size and shape of the beads were investigated to determine the optimum conditions for the study of the drug entrapment efficiency and the drug dissolution profiles.

The various sizes of chitosan beads from different preparations were shown in Table 4.2. The sizes of the beads for each formulation were observed using the light microscope. The size distribution depends on the diameter of a syringe needle, Formulations A to O, the beads were prepared by using syringe needles no. 18 and the beads sizes were in the range of 2.0-2.5 mm. When using a smaller diameter of syringe needles; no. 22 (Formulation P), no. 24 (Formulation Q). Beads from Formulation P showed the spherical shape, moreover the moderate diameter of the needle number 22 gave the suitable bead size. Because of this smaller size of beads should be the advantage in diffusing of TPP for crosslinking inside. Therefore the later preparation process of chitosan/PEG beads was performed by using the syringe needle no. 22 and the obtained beads sizes of chitosan/PEG were in the range of 1.92-2.12 mm, as presented in Table 4.3.

Table 4.2 The sizes of the chitosan beads obtained with various compositions as determined by a light microscope

Formulation	ratio of compositions			Tripolyphosphate		Time	Bead size ± S.D.
	CS	PEG	DS	conc. (%w/v)	pH value	(min.)	(mm.)
A0	1	0	0	-	-	20	2.18 ± 0.14
A1	1	0	1	-	-	20	2.37 ± 0.16
B	1	0	0	1	6.0	20	2.36 ± 0.12
C	1	0	0.25	1	6.0	20	2.44 ± 0.17
D	1	0	0.5	1	6.0	20	2.36 ± 0.20
E	1	0	0.75	1	6.0	20	2.40 ± 0.17
F	1	0	1	1	6.0	20	2.32 ± 0.05
G	1	0	1.5	1	6.0	20	2.30 ± 0.07
H	1	0	2	1	6.0	20	2.43 ± 0.14
I	1	0	3	1	6.0	20	nd
D	1	0	0.5	1	6.0	20	2.36 ± 0.20
J	1	0	0.5	5	6.0	20	2.37 ± 0.16
K	1	0	0.5	10	6.0	20	2.25± 0.08
K	1	0	0.5	10	6.0	20	2.25± 0.08
L	1	0	0.5	10	3.0	20	2.19 ± 0.24
M	1	0	0.5	10	8.0	20	2.34 ± 0.11
K	1	0	0.5	10	6.0	20	2.25± 0.08
N	1	0	0.5	10	6.0	30	2.24 ± 0.17
O	1	0	0.5	10	6.0	60	2.26 ± 0.06
N	1	0	0.5	10	6.0	30	2.24 ± 0.17
P*	1	0	0.5	10	6.0	30	1.94 ± 0.10
Q**	1	0	0.5	10	6.0	30	1.86 ± 0.17

[a] S.D. = Standard deviation

nd = not determined because beads could not form by the high viscosity solution.

* The beads formed by using a needle number 22.

** The beads formed by using a needle number 24.

Table 4.3 The sizes of the chitosan/PEG beads obtained with various compositions as determined by a light microscope

Formulation	ratio of compositions			Tripolyphosphate		Time (min.)	Bead size ± S.D.
	CS	PEG	DS	conc. (%w/v)	pH value		
P	1	0	0.5	10	-	30	1.94 ± 0.10
PEG0	1	1	0	10	6.0	30	2.01 ± 0.11
PEG1	1	0.25	0.5	10	6.0	30	2.04 ± 0.15
PEG2	1	0.5	0.5	10	6.0	30	1.92 ± 0.07
PEG3	1	1	0.5	10	6.0	30	1.95 ± 0.11
PEG4	1	1.5	0.5	10	6.0	30	2.06 ± 0.13
PEG5	1	0.5	0.25	10	6.0	30	1.96 ± 0.12
PEG6	1	0.5	0.5	10	6.0	30	2.01 ± 0.09
PEG2	1	0.5	1	10	6.0	30	1.92 ± 0.07
PEG7	1	0.5	1.5	10	6.0	30	2.09 ± 0.08

[a] S.D. = Standard deviation

4.2.2 Morphology of the beads

The surface topography, size and shape of the dried beads were observed using the scanning electron microscopy (SEM). The effects of various ratios of chitosan/PEG, DS content and various conditions of the coagulant solutions were investigated. The microscopic images were taken in three magnifications for each formulation as illustrated in Figures 4.1-4.8.

Effects of a cross-linking agent

Figure 4.1 shows the photomicrographs of the beads produced by various types of coagulant solutions: (A) (5%(w/v) NaOH and (B) 1%(w/v) TPP. These beads were not completely spherical. The different coagulant solutions gave different surface of chitosan beads. Especially, the surface of the beads from formulation A0, which were prepared from only CS using 5% (w/v) NaOH as a coagulant, had rougher and more folded than the others (Figure 4.1) because of the network shrinking during a freeze drying process. Although, using 1% (w/v) TPP coagulant gave the smoother surface beads but more fragile, probably due to the low concentration of TPP solution could not entirely form a polyelectrolyte complex with chitosan. Therefore, pure chitosan network inside the beads showed the brittle bead character. From Figure 4.1, the cross-sections of both of chitosan beads, prepared by 5% NaOH and 1% TPP, are also different. Formulation A0 showed the large porous network inside, because OH^- groups could deprotonate NH_4^+ site on CS and the porous network having only NH_3 groups of chitosan was formed. In contrast, the cross-section photomicrographs of Formulation B, PO_{10}^{3-} group could form a polyelectrolyte complex with NH_4^+ site and the highly porous, coral like network was presented. Thus, TPP should be more suitable coagulant because the structure of polyelectrolyte complex network could entrap more drugs.

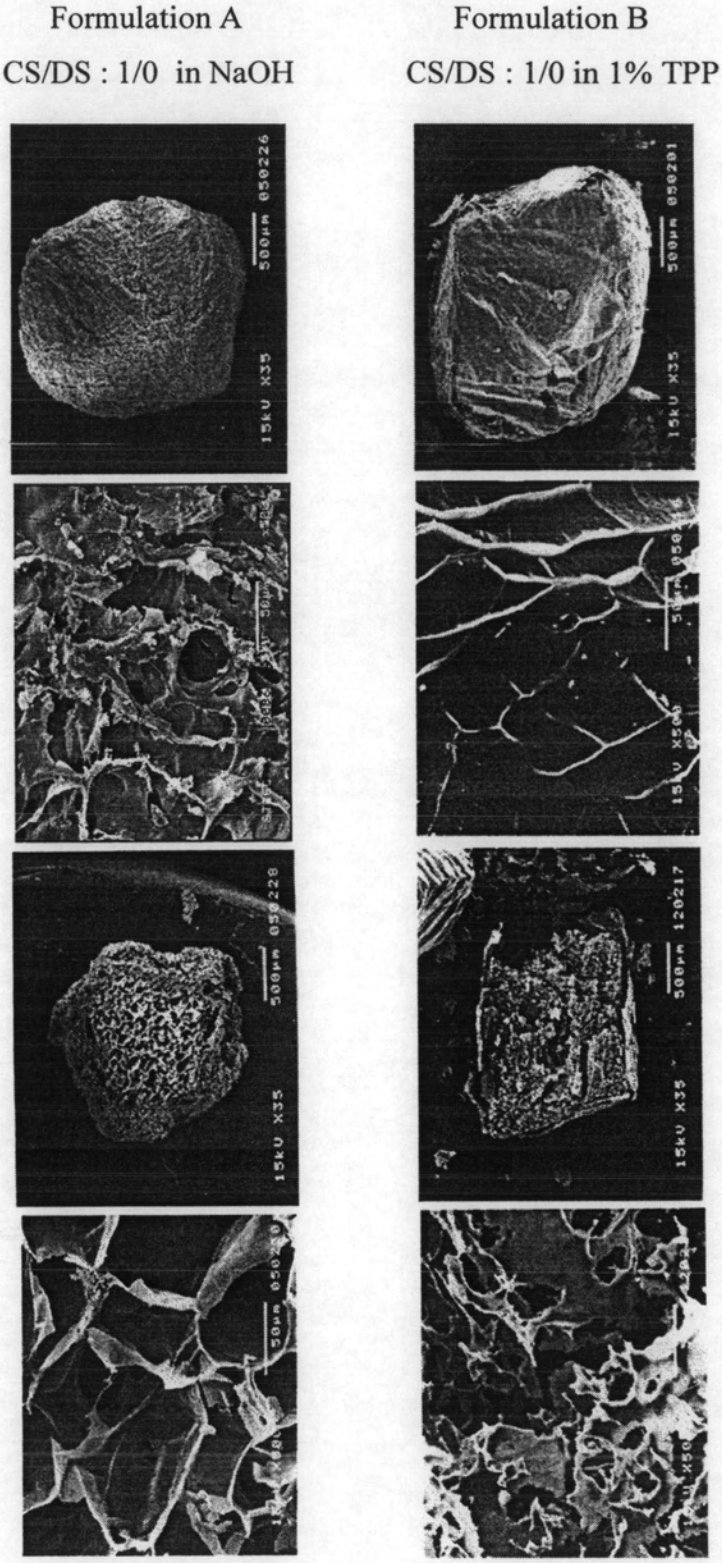


Figure 4.1 Scanning electron photomicrographs of the beads (x35), surface (x500), cross-section beads (x35) and network inside bead (x500) prepared in 5%(w/v) NaOH and 1%(w/v) TPP in formulation A0 and B, respectively.

Effect of chitosan to DS ratio.

Figure 4.2 shows photomicrographs of formulation B to H, all formulations were prepared in various chitosan to DS ratios in 1% TPP.

From Figure 4.2, in all formulations, the beads were prepared in TPP solution showed the rough surface of the chitosan-TPP network formation. The cross-section photomicrographs in Figure 4.2 showed that the increase of DS content, formulation B to H, gave higher porosity of network inside the beads. As the results of ionic interactions between the NH_4^+ groups of chitosan and the COO^- of DS, the strong network was formed as increasing the amount of drug contents.

Formulation D (2/1: chitosan /DS) was the most suitable condition because it presented a high percentage drug entrapment efficiency in Table 4.3, thus this formulation condition was used for further experiments.

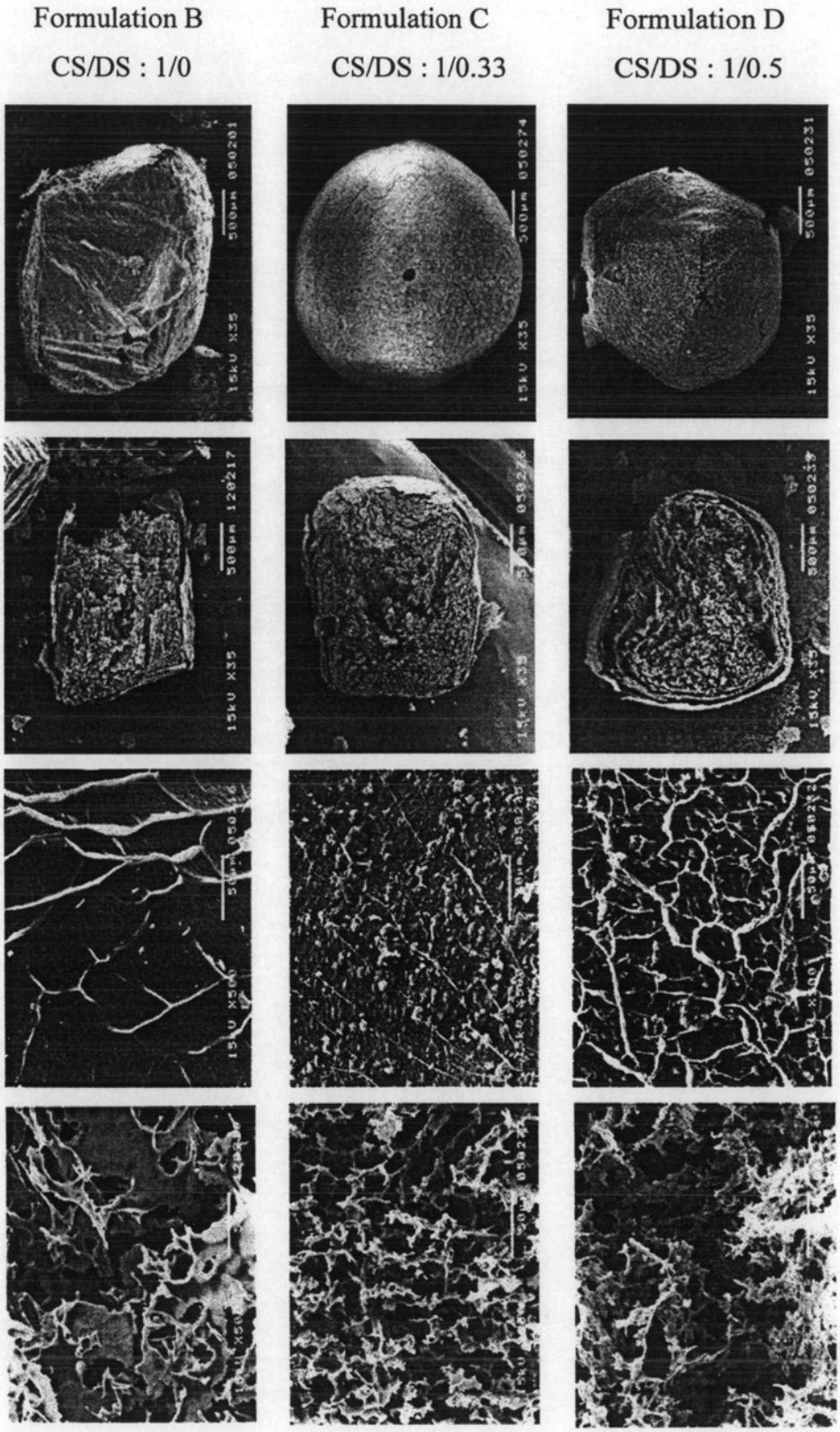


Figure 4.2 Scanning electron photomicrographs of the beads (x35), surface (x500), cross-section beads (x35) and network inside bead (x500) prepared at 1% TPP with various ratios of chitosan/DS (Formulation B: 1/0, C: 1/0.33, D: 1/0.5)

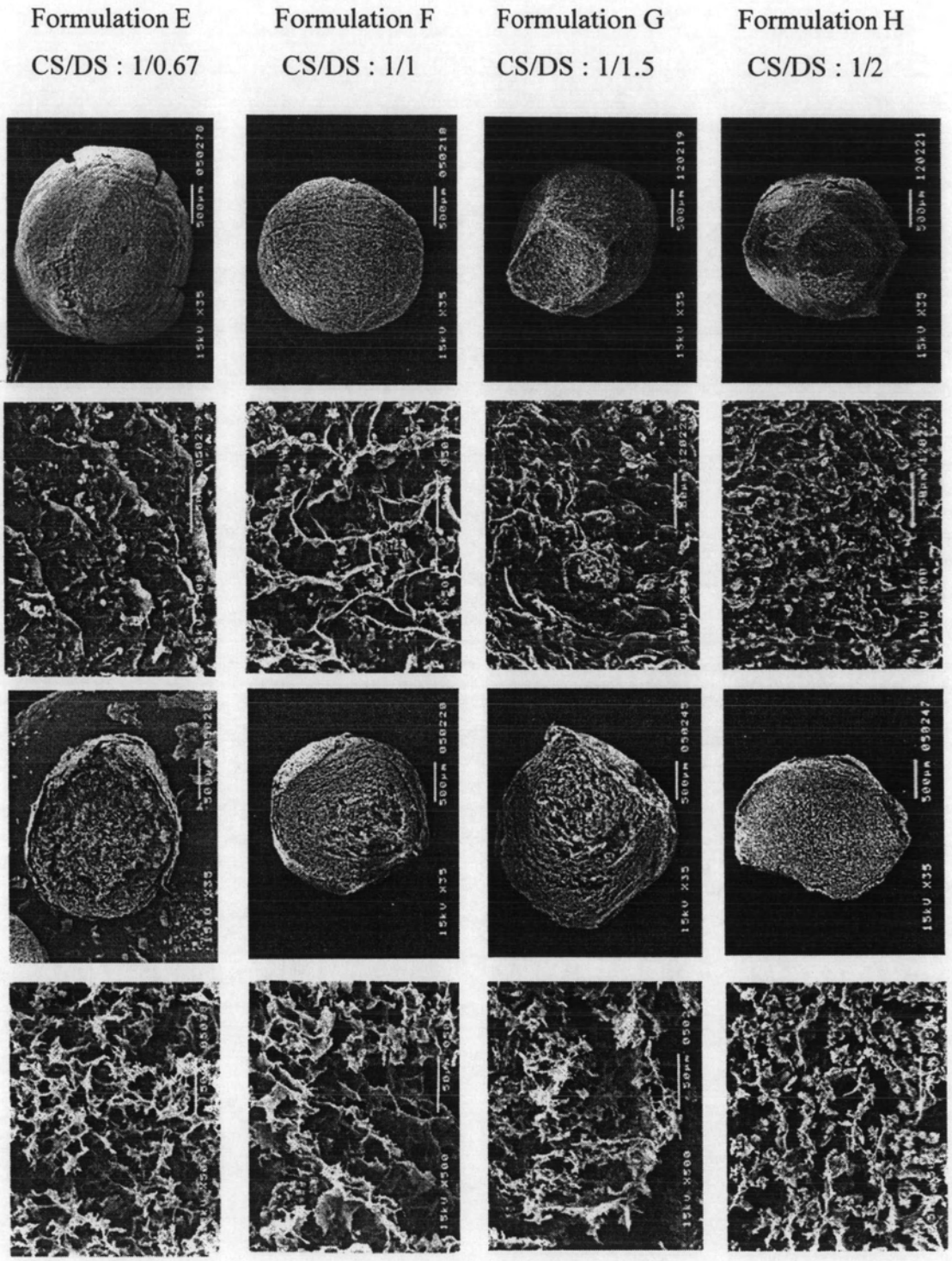


Figure 4.2 (continue) Scanning electron photomicrographs of the beads (x35), surface (x500), cross-section beads (x35) and network inside bead (x500) prepared at 1% TPP with various ratios of chitosan/DS (Formulation E: 1/0.67, F: 1/1, G: 1/1.5, H: 1/2)

Effect of the concentration of TPP

However the hydrogel beads from Formulation D still presented the fragility, so the coagulant conditions should be varied. Figure 4.3 illustrated photomicrographs of 2/1 chitosan to DS ratio prepared in various conditions of TPP 1%, 5% and 10% (w/v) were referred as Formulation D, J and K, respectively. All conditions of TPP were adjusted to pH value 6.0 by adding conc. HCl acid. Because of the higher amount of $P_3O_{10}^{5-}$ ions from the higher concentration of TPP, a firm polyelectrolyte complex with chitosan was obtained. So the beads produced in 10% of TPP, like Formulation K, formed the fine porous and the strong networks, resulting in a good drug entrapment and may give advantage in controlling the drug release. Thus, 10% (w/v) of TPP was the optimum concentration for the chitosan bead preparations.

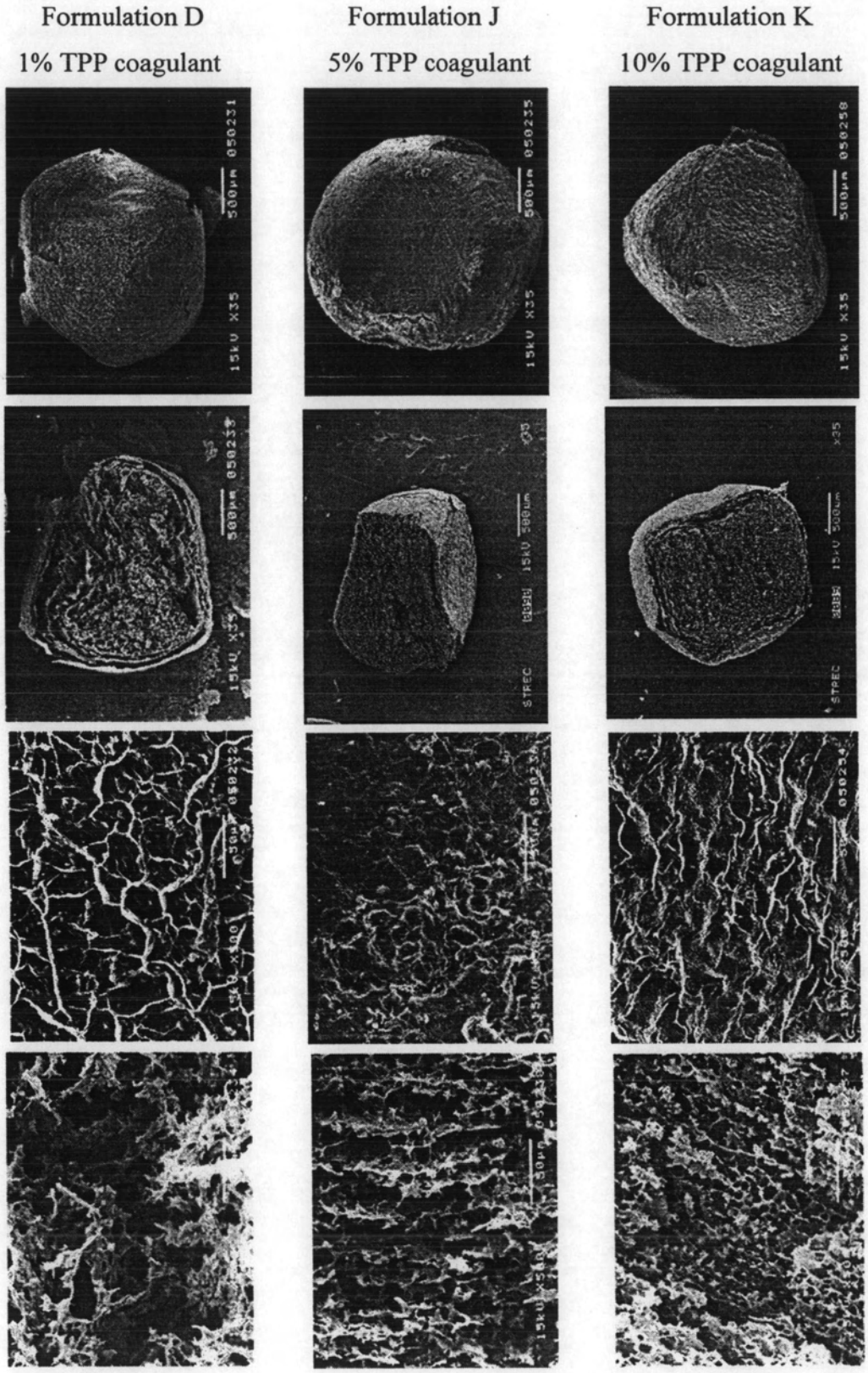


Figure 4.3 Scanning electron photomicrographs of the beads (x35), surface (x500), cross-section beads (x35) and network inside bead (x500) prepared at various concentration of TPP (Formulation D: 1%TPP, J: 5%TPP and K: 10% TPP)

Effect of the pH value of TPP solution

Figure 4.4 shows the photomicrographs of chitosan beads, surface, cross-section and network inside beads produced by various pH values of sodium TPP solutions at pH 3.0, pH 6.0 and pH 8.0 (Formulation L, K and M, respectively) in different magnifications.

Chitosan beads and cross-section morphology from Figure 4.4 showed the different characters at the different conditions of TPP. Hence, at pH 3.0, TPP consists of only $P_3O_{10}^{5-}$ anions and freely interacted with $-NH_3^+$ sites of the chitosan, so at this condition, polymer network inside the chitosan beads were formed by ionic cross-linking of $P_3O_{10}^{5-}$ and high density of the polyelectrolyte complex network was formed. On the other hand, at higher pH conditions (6.0 and 8.0) of TPP, $P_3O_{10}^{5-}$ and OH^- were released from TPP. There were the competitions between those anions for interacting with chitosan. Thus the networks inside chitosan beads consisted of both ionic cross-linking networks of $P_3O_{10}^{5-}$ and deprotonated networks of OH^- to chitosan. The photomicrograph of cross-section of Formulation M, prepared at TPP pH 8.0, showed the hollow porous network, especially at the center of bead like they were formed in NaOH, but different from other formulations. On account of the deprotonation reaction of the small molecules, OH^- could form bead faster than $P_3O_{10}^{5-}$ and creating a large porous network [32].

For the above reasons, the 10% TPP at pH 6.0 is the most suitable pH condition. Because its polymer networks inside the beads were not too dense and not too hollow. Moreover, this condition is quite neutral and is not toxic. Hence it could be applied to use in pharmaceutical fields.

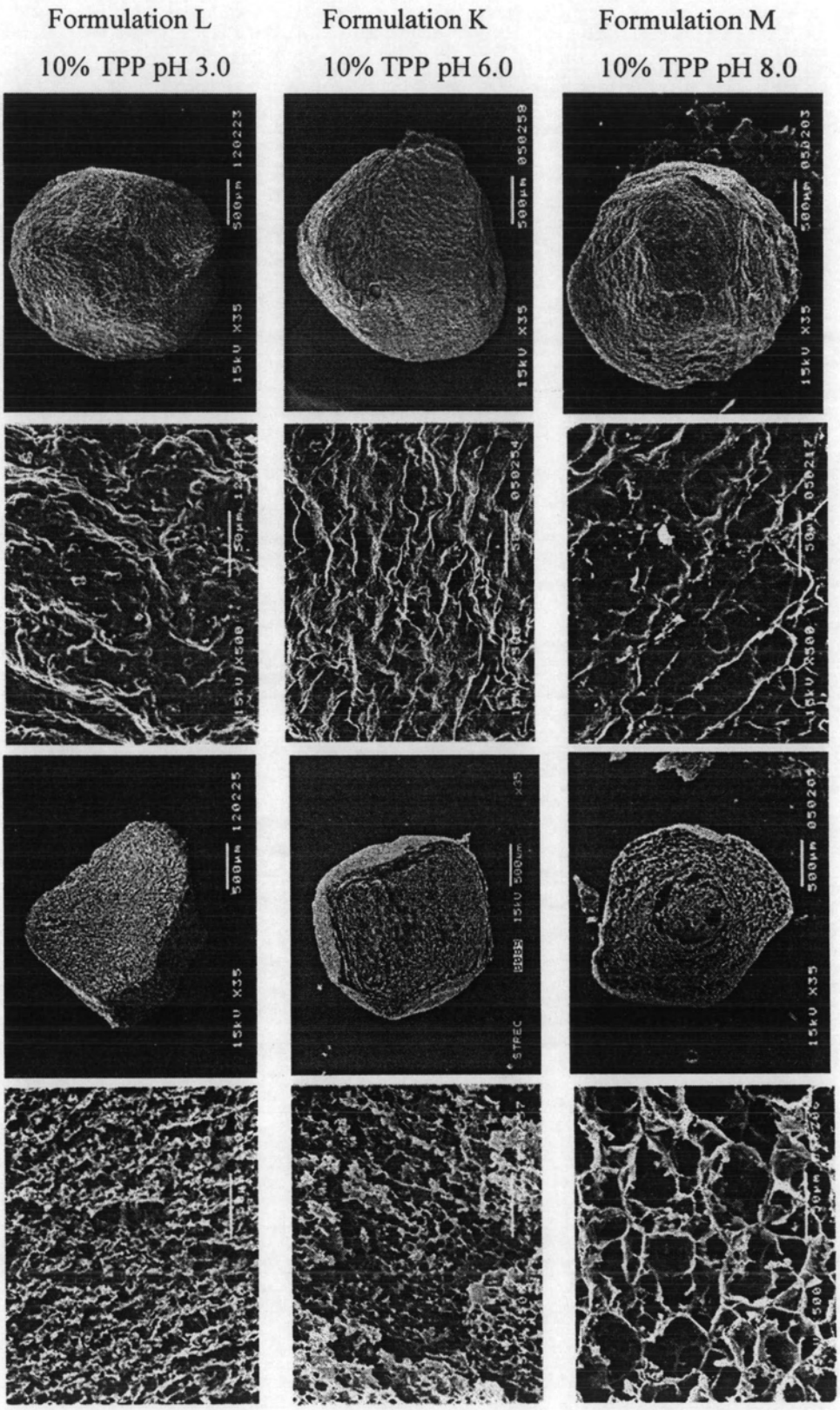


Figure 4.4 Scanning electron photomicrographs of the beads (x35), surface (x500), cross-section beads (x35) and network inside bead (x500) prepared with various pH values of TPP coagulant (Formulation L: pH 3, K: pH 6 and M : pH 8).

Effect of cross linking time

According to, the ionic interactions between chitosan and TPP depended on the diffusion rate of $P_3O_{10}^{5-}$ anions into chitosan droplet. Therefore, cross-linking time of beads should be another factor that was effective in chitosan-TPP beads forming. As shown in Figure 4.5, Formulations K, N and O were cross-linked for 20, 30 and 60 minutes, respectively. All types of chitosan-TPP beads gave the high porous and dense network inside, because the cross-linking structure was formed. TPP diffused from outside into the core of the droplet. As the result, the cross-linking time was an important factor in forming the network, which might have a slightly different structure.

In addition, the density of the cross-linking network inside bead was increased while increasing the cross-linking time. Although the longer time of cross-linking was suitable for the complete ionic interactions inside the beads, the too high density of the network might be the problem of the poor drug entrapment. Therefore the moderate cross-linking time, 30 minutes, was the most suitable crosslinking time in chitosan bead preparations for drug entrapment efficiency and drug controlled release profile.

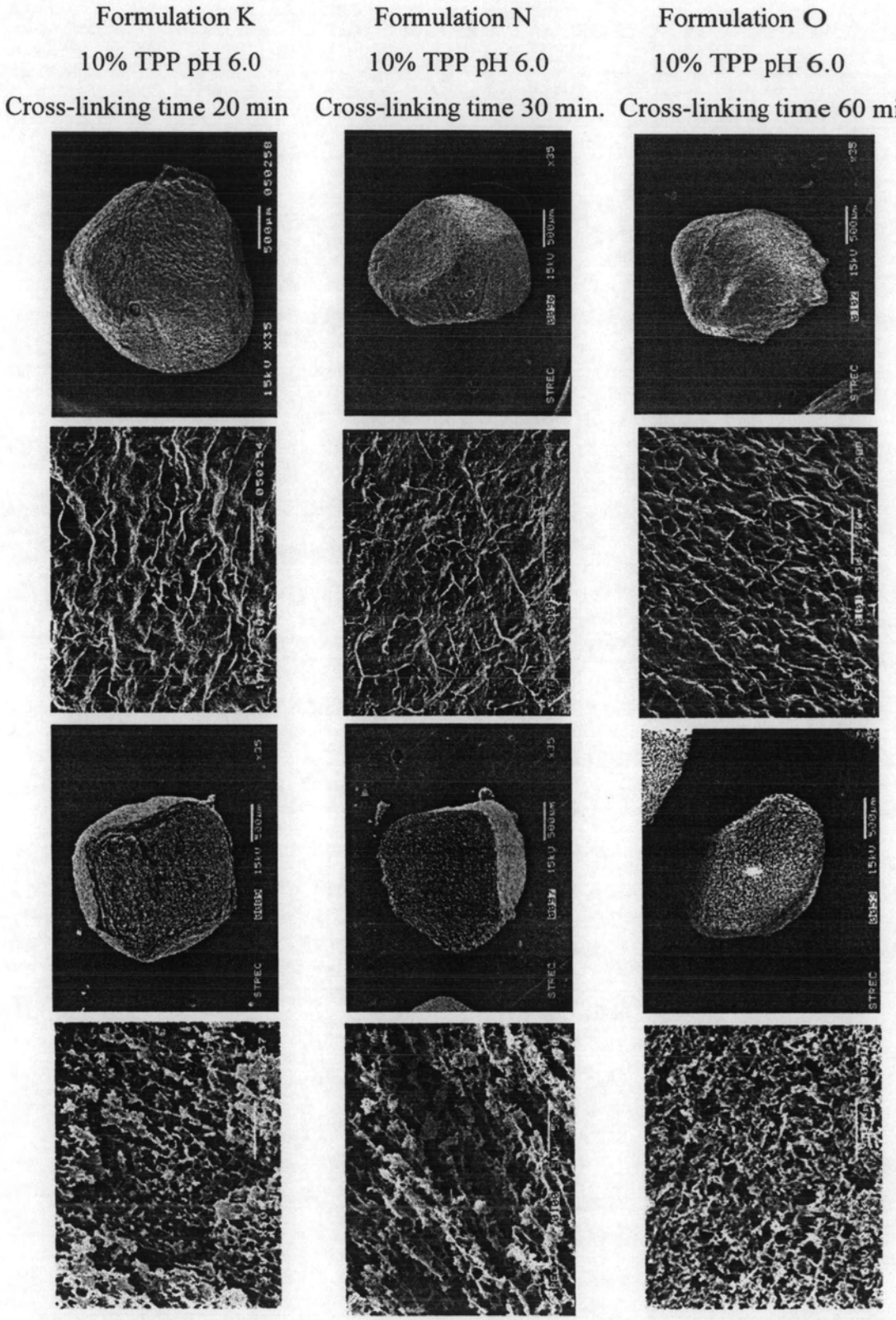


Figure 4.5 Scanning electron photomicrographs of the beads (x35), surface (x500), cross-section beads (x35) and network inside bead (x500) prepared with various cross-linking times; (Formulation K : 20, N: 30 and O : 60 minutes).

Effect of chitosan bead size

The beads sizes of chitosan were important for application in pharmaceutical field. In general, the beads with small sizes would facilitate in the administration. Thus the various sizes of chitosan beads were studied in Figure 4.6. At first, the biggest size of the syringe needle number 18 (formulation N) was used for the bead preparations. Later, the sizes of the beads were improved on the smaller sizes by using syringe needles number 22 (formulation P) and 24 (formulation Q). From Figure 4.6, all formulations of chitosan beads presented the same surface and structure of network inside beads, therefore the results of drug entrapment and controlled release profiles should be concerned.

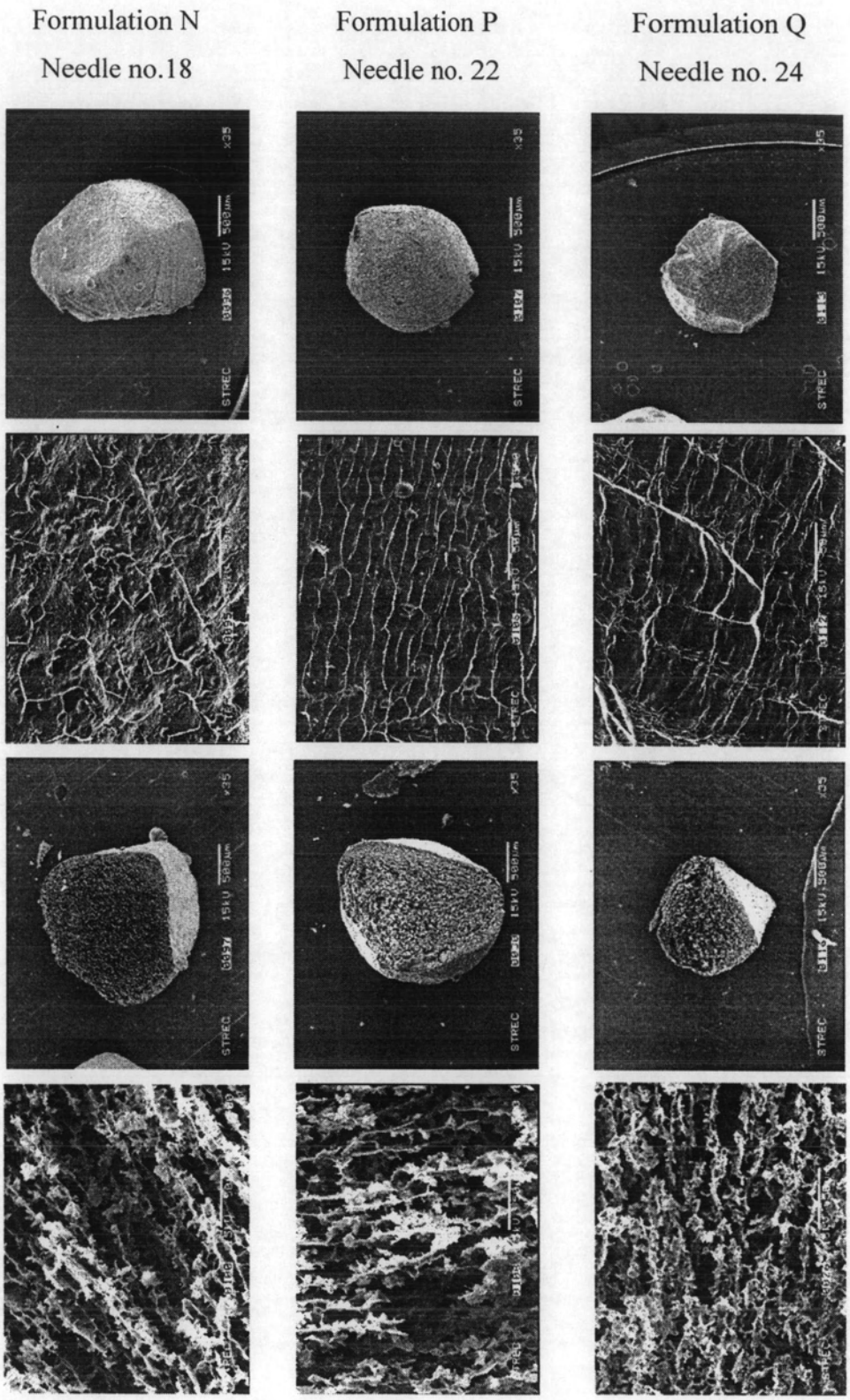


Figure 4.6 Scanning electron photomicrographs of the beads (x35), surface (x500), cross-section beads (x35) and network inside bead (x500) prepared with various sizes of the beads by using the different syringe needles ; (Formulation N : No.18, P: No. 22 and O : No. 24).

Table 4.4 The sizes of the chitosan/PEG beads obtained with various compositions as determined by a light microscope

Formulation	ratio of compositions			Tripolyphosphate		Time (min.)	Bead size ± S.D.
	chitosan	PEG	DS	conc. (%w/v)	pH value		
P	1	0	0.5	10	-	30	1.94 ± 0.10
PEG0	1	1	0	10	6.0	30	2.01 ± 0.11
PEG1	1	0.25	0.5	10	6.0	30	2.04 ± 0.15
PEG2	1	0.5	0.5	10	6.0	30	1.92 ± 0.07
PEG3	1	1	0.5	10	6.0	30	1.95 ± 0.11
PEG4	1	1.5	0.5	10	6.0	30	2.06 ± 0.13
PEG5	1	0.5	0.25	10	6.0	30	1.96 ± 0.12
PEG6	1	0.5	0.5	10	6.0	30	2.01 ± 0.09
PEG2	1	0.5	1	10	6.0	30	1.92 ± 0.07
PEG7	1	0.5	1.5	10	6.0	30	2.09 ± 0.08

[a] S.D. = Standard deviation

Effect of chitosan/PEG ratio

Once the optimum condition for chitosan-TPP bead preparation was achieved, some properties of chitosan beads, such as swelling and controlled drug release behavior, should be improvable. Polyethylene glycol (PEG) is the polymer that could reinforce the chitosan-TPP beads and improve those properties.

Formulation P was chitosan beads which contained only DS, PEG0 was chitosan beads contained only PEG and Formulation PEG3 was chitosan beads contained both DS and PEG. The formulas of those formulations were exhibited in Table 4.3 and the photomicrographs of those formulations were illustrated in Figure 4.7. Because DS could directly form the networks with chitosan, therefore both Formulations P and PEG3 presented the dense networks inside beads. Therefore the existence of the DS in beads was important in bead forming networks. The comparison of Formulations P and PEG3, in Figure 4.7, showed that the addition of PEG into chitosan hydrogel could give the little increase of the dense networks. As the results, the advantages of using PEG would be determined in the in vitro release study at Figures 4.37-4.38 and Table C1 (Appendix C).

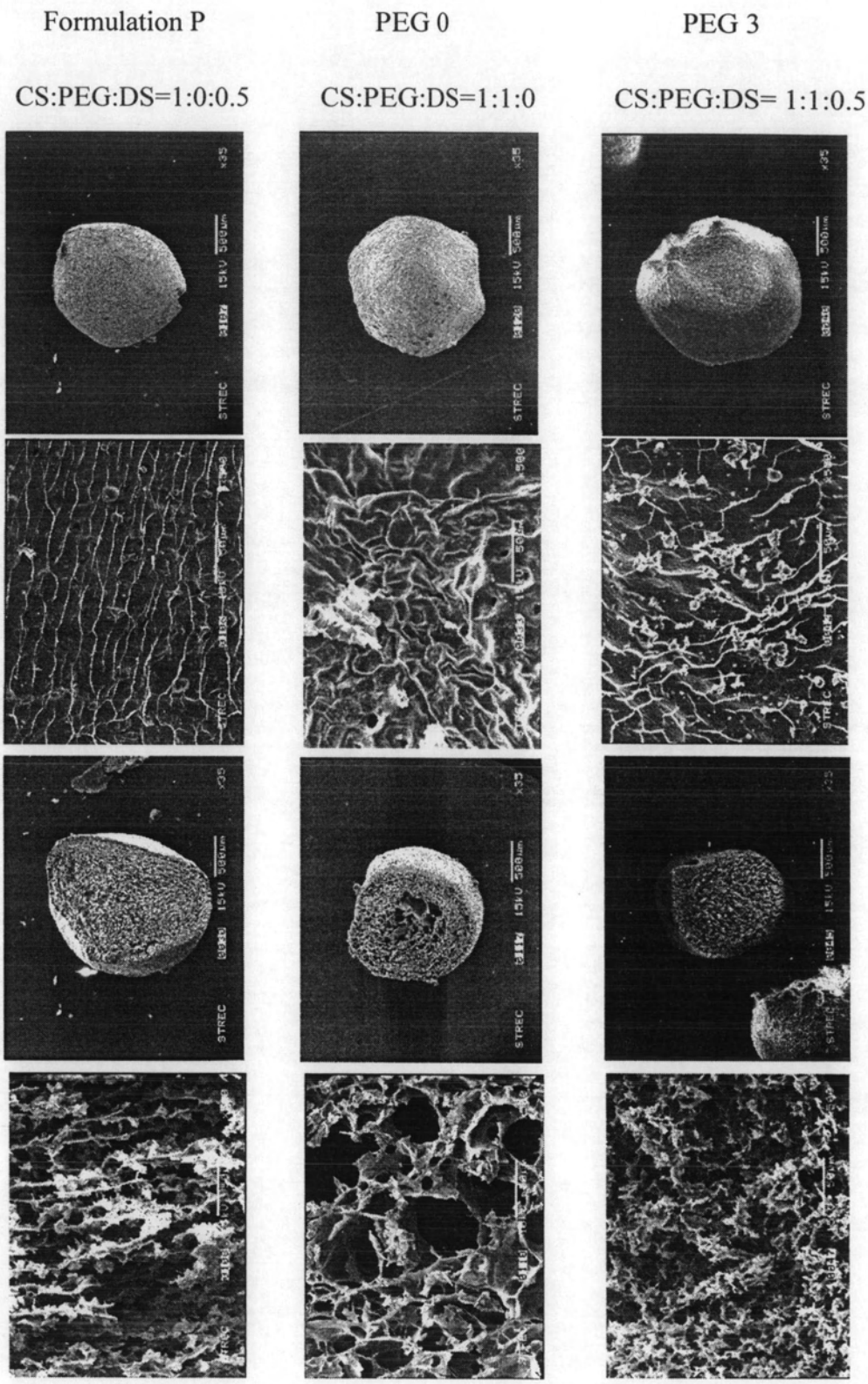


Figure 4.7 Scanning electron photomicrographs of the beads (x35), surface (x500), cross-section beads (x35) and network inside bead (x500) prepared with various ratios of chitosan/PEG (**Formulation P**: CS/PEG/DS = 1/0/0.5, **PEG0** : CS/PEG/DS = 1/0/0 and **PEG3** : CS/PEG/DS =1/1/0.5).

Figure 4.8 illustrated the photomicrographs of chitosan/PEG beads, prepared by various chitosan/PEG ratios (1/0.25, 1/0.5, 1/1 and 1/2). Moreover, All formulations were fixed the DS content at 50% (w/w) of chitosan (chitosan/DS:1/0.5). Each formulation of chitosan/PEG beads presented the tight network inside. As the result of the complete interaction between chitosan, TPP and DS, so chitosan beads reinforced by PEG showed the little increase of the dense network. Thus the suitable ratio of chitosan/PEG in formulation should be obtained from the results % release of drug in Table C1 (Appendix C). From the highest %release of DS (92.66%) was obtained therefore the most suitable formulation in the chitosan/PEG ratio should be Formulation PEG2.

After the suitable proportion in 1/0.5 of chitosan/PEG was obtained, the various DS ratios would be studied. Figure 4.8, the DS content was added into the formulations and the chitosan/PEG ratio was fixed at 1/0.5 before varying Formulation PEG5, PEG2, PEG6 and PEG7, at displayed in Table 4.5.

When the DS was increased in beads, an excess DS powder was found at the surface. Therefore the high DS content might be provided the burst release of drug due to the rapidly lost of drug at the surface. The optimum ratio of chitosan/PEG/DS should be 1/0.5/0.5 which gave the best behavior in drug controlled release as shown in Formulation PEG2 in Table C1 (Appendix C).

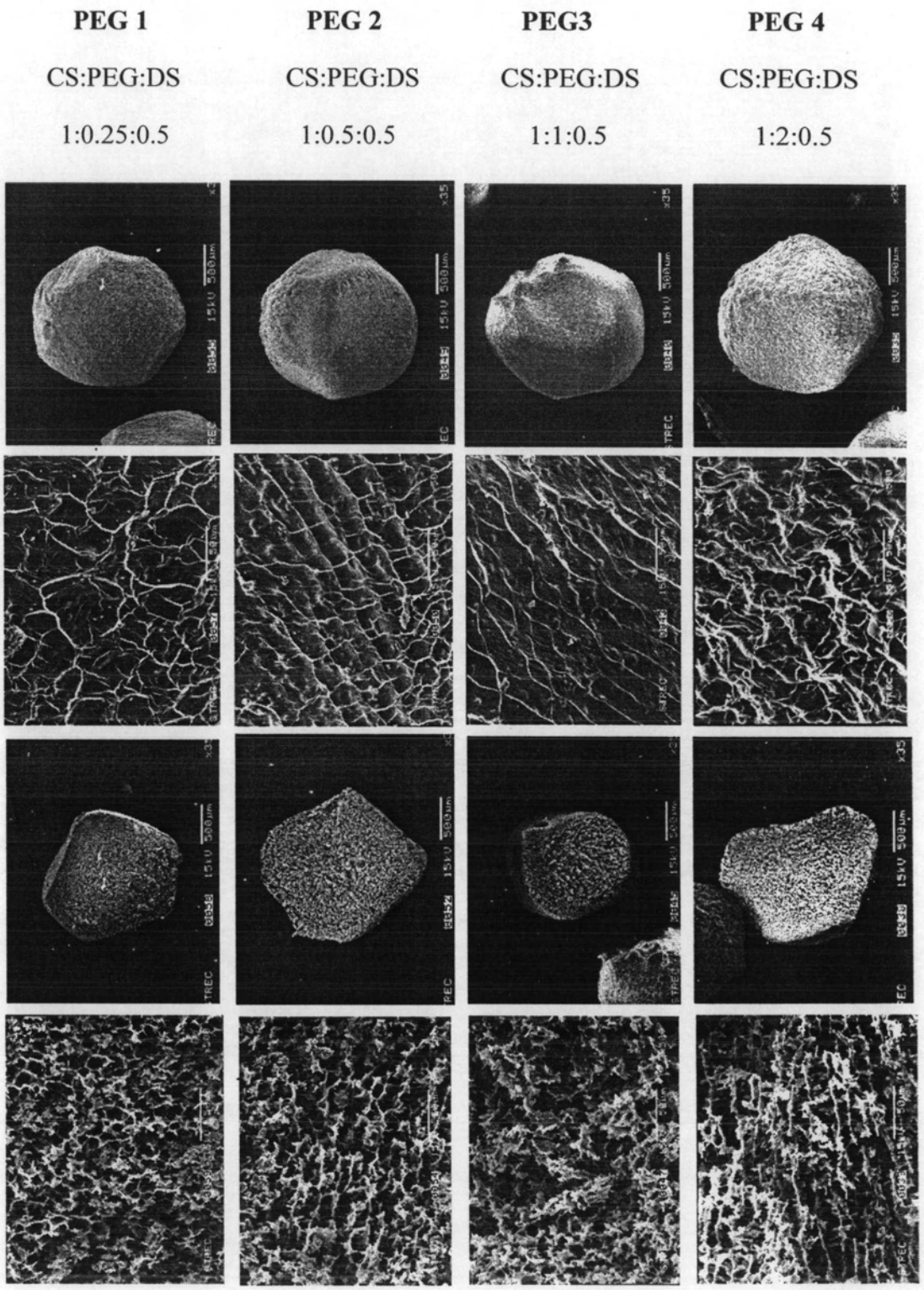


Figure 4.8 Scanning electron photomicrographs of the chitosan/PEG beads (x35), surface (x500), cross-section beads (x35) and network inside bead (x500) prepared with various chitosan/PEG ratios at the same drug content; (Formulation **PEG1** : CS/PEG=1:0.25, **PEG2** : CS/PEG=1:0.5, **PEG3** : CS/PEG=1:1 and **PEG 4** : CS/ PEG=1:2).

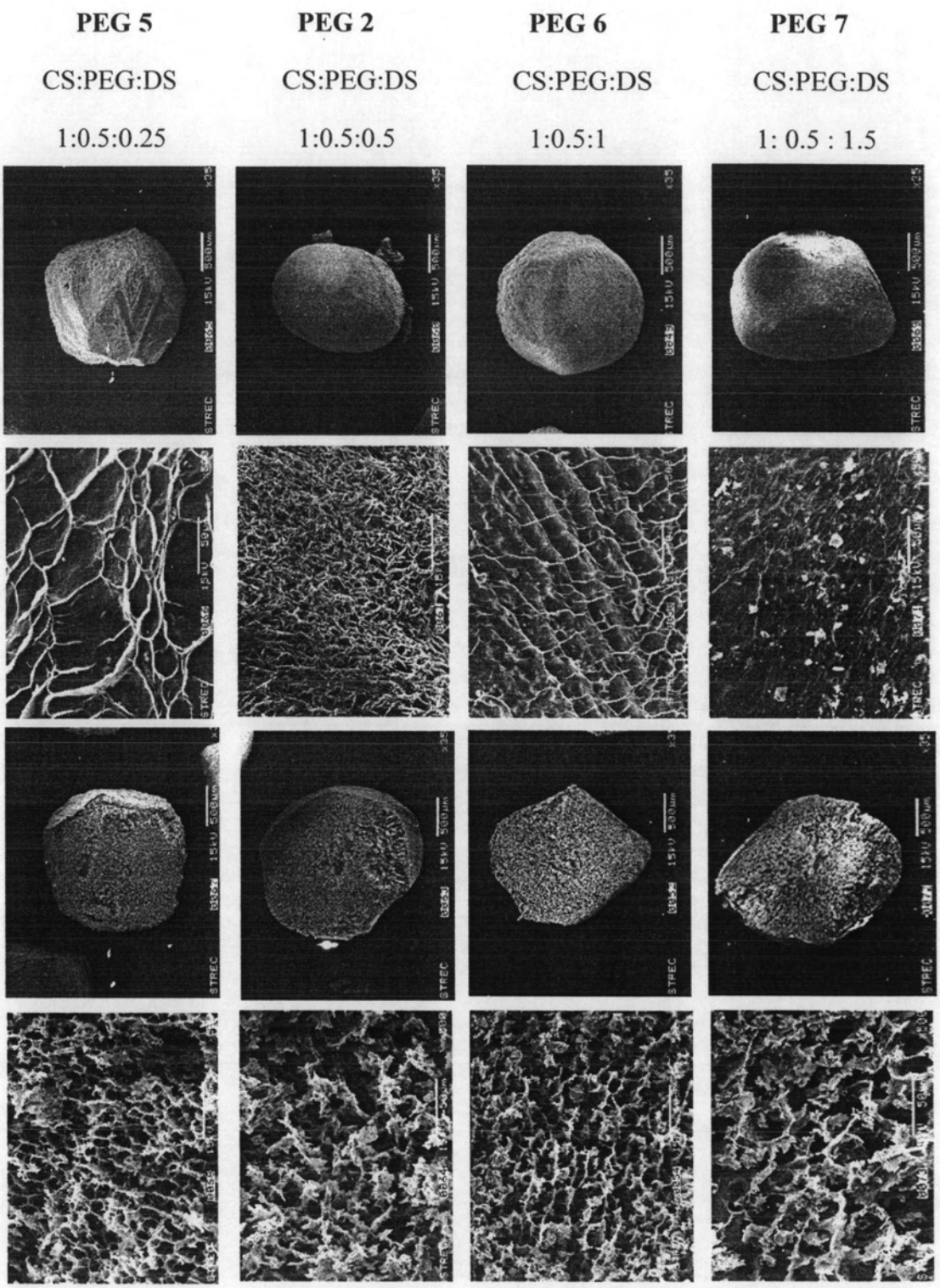


Figure 4.9 Scanning electron photomicrographs of the chitosan/PEG beads (x35), surface (x500), cross-section beads (x35) and network inside bead (x500) prepared with various chitosan/DS ratios by fixing the CS/PEG ratio at 1:0.5; (Formulation **PEG5**: CS/PEG/DS =1/0.5/0.25, **PEG6** : CS/PEG/DS =1/0.5/0.5, **PEG**2 : CS/PEG/DS =1/0.5/1, **PEG7** : CS/PEG/DS =1/0.5/2.

4.2.3 Fourier transform infrared spectroscopy (FT-IR)

The IR spectrums of the tested samples are shown in Figures 4.10-4.13. Figure 4.10 (a) shows the IR spectrum of chitosan. The peaks were observed at 1592, 1652, 2872 and 3422 cm^{-1}. The peak at 1592 cm^{-1} resulted from N-H bending of primary amide group. The IR absorption peak at 1652 and 2872 cm^{-1} correspond to C=O of amide groups and $-CH_3$ stretching respectively [36]. These two peaks represent a fraction of chitin that was not hydrolyzed. The broad absorption band around 3422 cm^{-1} resulted from the stretching vibration of O-H bonded to N-H stretching.

Figure 4.10 (b) shows the IR spectrum of TPP. The principle peaks were observed at 1162 and 1211 cm^{-1} resulted from P–O stretching of $-P_3O_{10}^{5-}$ anions. The –OH stretching was found at 3444 cm^{-1}.

Figure 4.10 (c) shows the IR spectrum of chitosan-TPP beads. Because of the ionic interaction between chitosan and TPP, the spectrum showed a new absorption peak of C=O at 1636 and NH_3 at 1532 cm^{-1} which displaces absorption peaks at 1652 and 1592 cm^{-1}, respectively. For absorption peaks correspond to P–O were shifted from 1162 and 1211 cm^{-1} to 1151 and 1086 cm^{-1}, respectively.

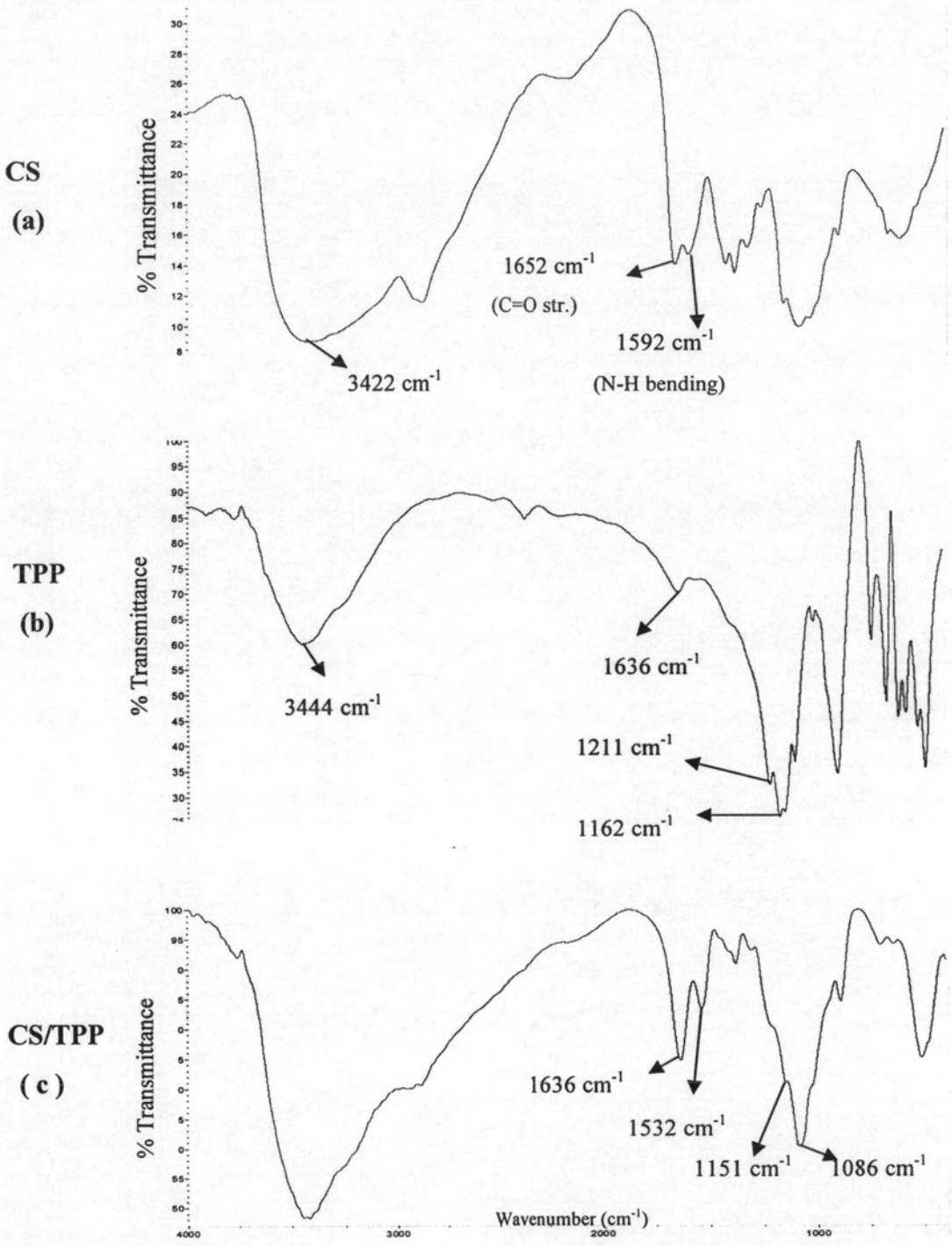


Figure 4.10 FT-IR of (a) chitosan (CS), (b) sodium tripolyphosphate (TPP) and (c) CS/TPP beads.

Figure 4.11 (b) shows the IR spectrum of DS. The principle peaks were observed at wave numbers 743, 765, 1293, 1302, 1391, 1451and 1575 cm^{-1}. The peaks at 743 and 765 cm^{-1} resulted from C-H out of plane bending. The IR absorption bands at 1293 and 1302 cm^{-1} resulted from C-N stretching and the peak 1575 cm^{-1} resulted from C=C stretching combined with C=O stretching of carboxylate group respectively.

Figure 4.11 (c) displays the IR spectrum of DFH. DS can be converted into DFH (diclofenac acid) in an acidic solution. The peak of stronger C=O group of carboxylic acid was found at 1690 cm^{-1} and C=C stretching also presented at 1575 cm^{-1}. The peak for free –OH group was found at 3313 cm^{-1}.

Figure 4.11 (d) shows the IR spectrum of CS/TPP loaded DS beads. The peak of C=O carboxylic acid also presented at 1690 cm^{-1} and the C=O peak of amide group was observed at 1636 cm^{-1}.

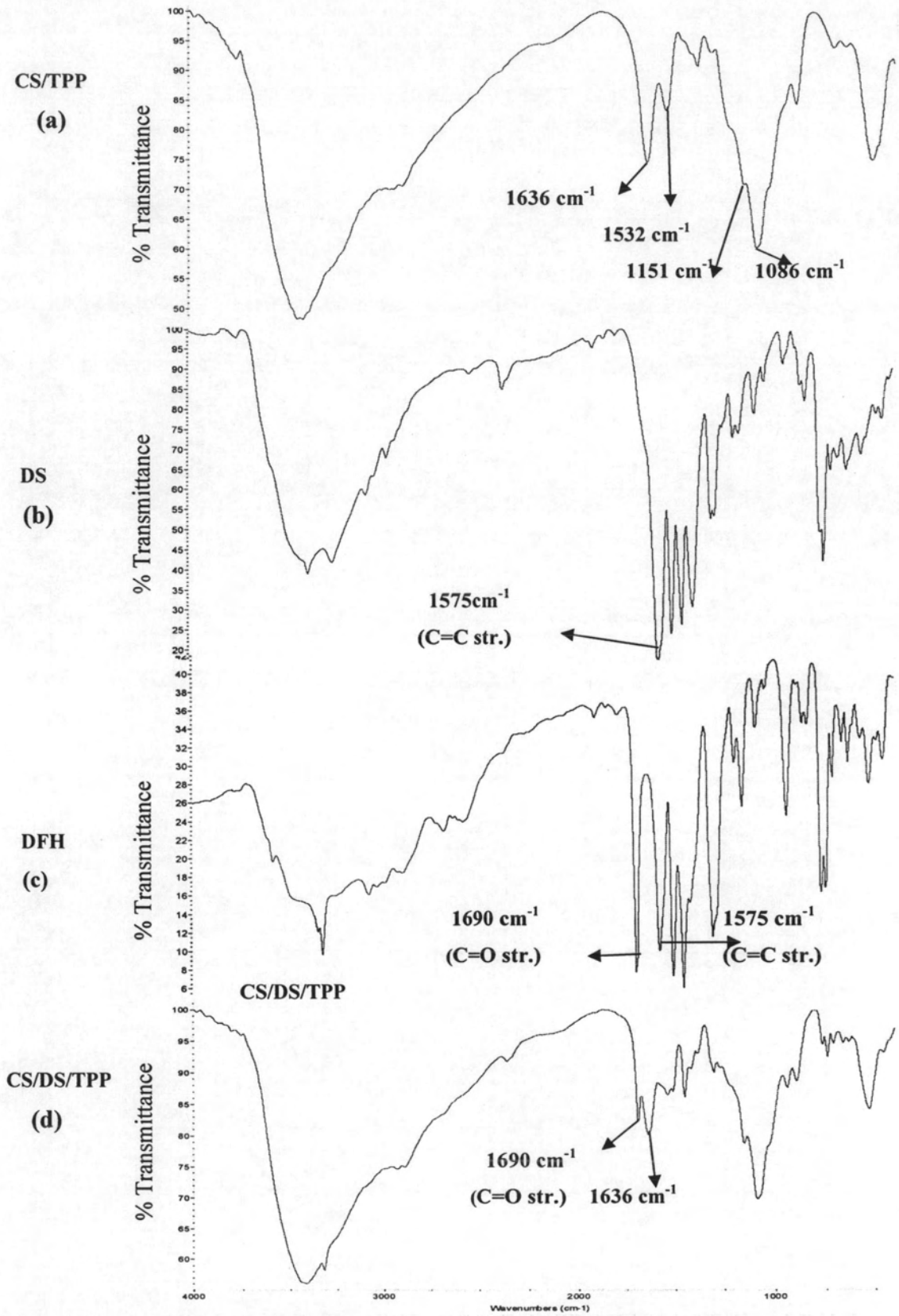


Figure 4.11 FT-IR Spectrum of (a) CS/TPP, (b) sodium diclofenac (DS), (c) diclofenac acid (DFH) and (d) CS/DS/TPP beads.

Figure 4.12 (b) shows the IR spectrum of PEG. The absorption band at 1102 cm^{-1} of PEG was attributed to the bending vibration of C-O, and two absorption bands at 1347 and 2878 cm^{-1} were attributed to the bending vibration and stretching vibration of C-H, respectively. Finally, the wide absorption band around 3421cm^{-1} was due to the stretching vibration of O-H bonded to N-H [6].

Figure 4.12 (c) showed the IR spectrum of CS/PEG beads contained DS. It can be seen that the characteristic absorption bands at 1097, 1641, 1538 and 2883 cm^{-1} which were shifted from the lower wave number 1086, 1636, 1632 and 2878cm^{-1} of pure PEG, respectively. Moreover, there were no obvious chemical reaction between chitosan and PEG. All those results indicated that PEG had only strong hydrogen bonds and ionic bonds with the matrix.

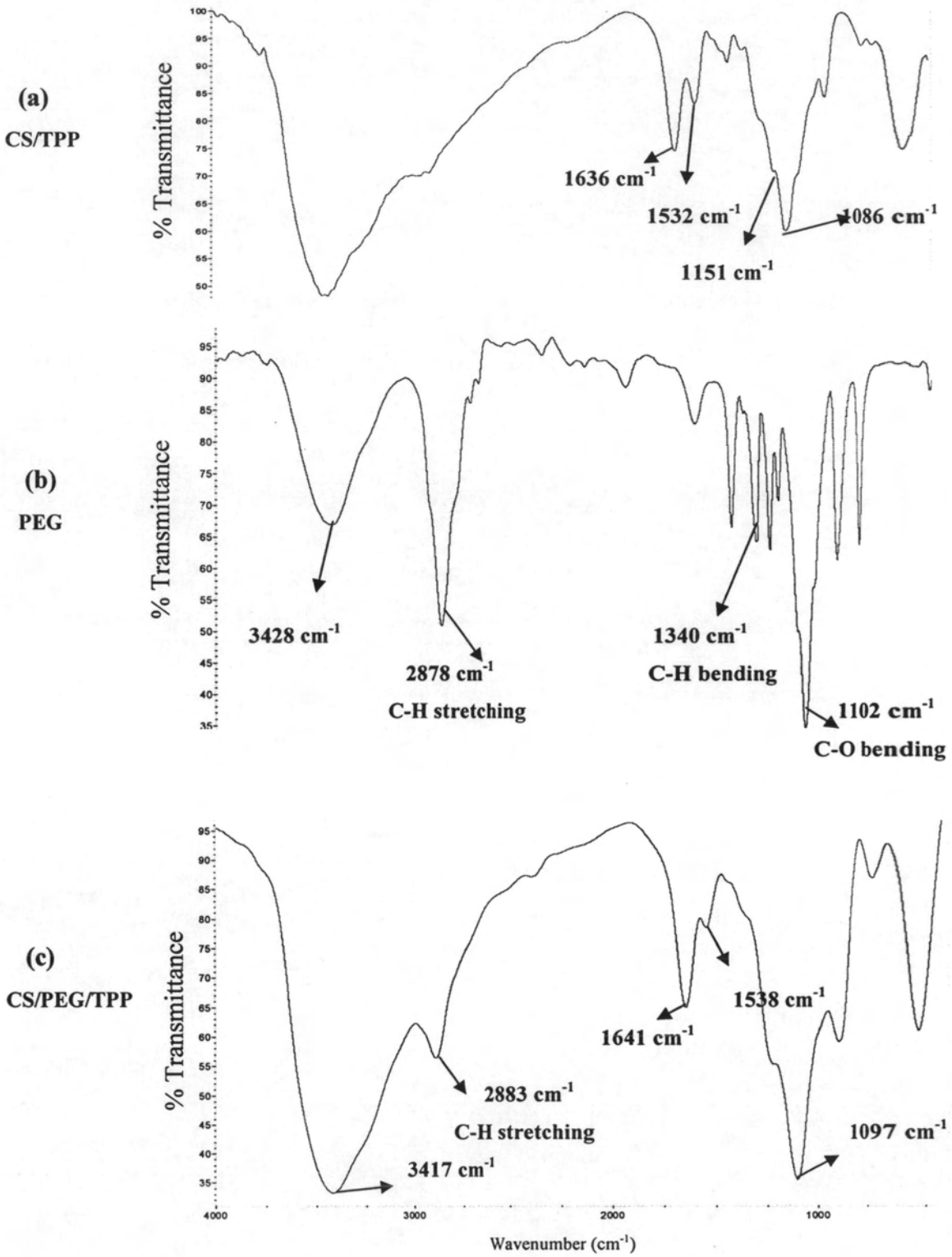


Figure 4.12 FT-IR spectrum of (a) CS/TPP beads, (b) polyethylene glycol and (c) CS/PEG/TPP beads.

Figure 4.13 (a)-(d) illustrate the FT-IR spectrum of the various ratios of DS loaded in 1/0.5 CS/PEG. The increasing intensity of peaks C=O stretching of carbonyl group at the wave number of 1690 cm^{-1} and the N-H stretching at about 3313 cm^{-1} from DS were found by increasing the amount of DS.

Figure 4.13 (e) presents the FT-IR spectrum of CS/PEG/DS/GD beads, chitosan/PEG cross-linked with glutaraldehyde. The two absorption bands at 1685 and 1641 cm^{-1} were attributed to C=O stretching of carboxylate combined with the C=O stretching of aldehyde.

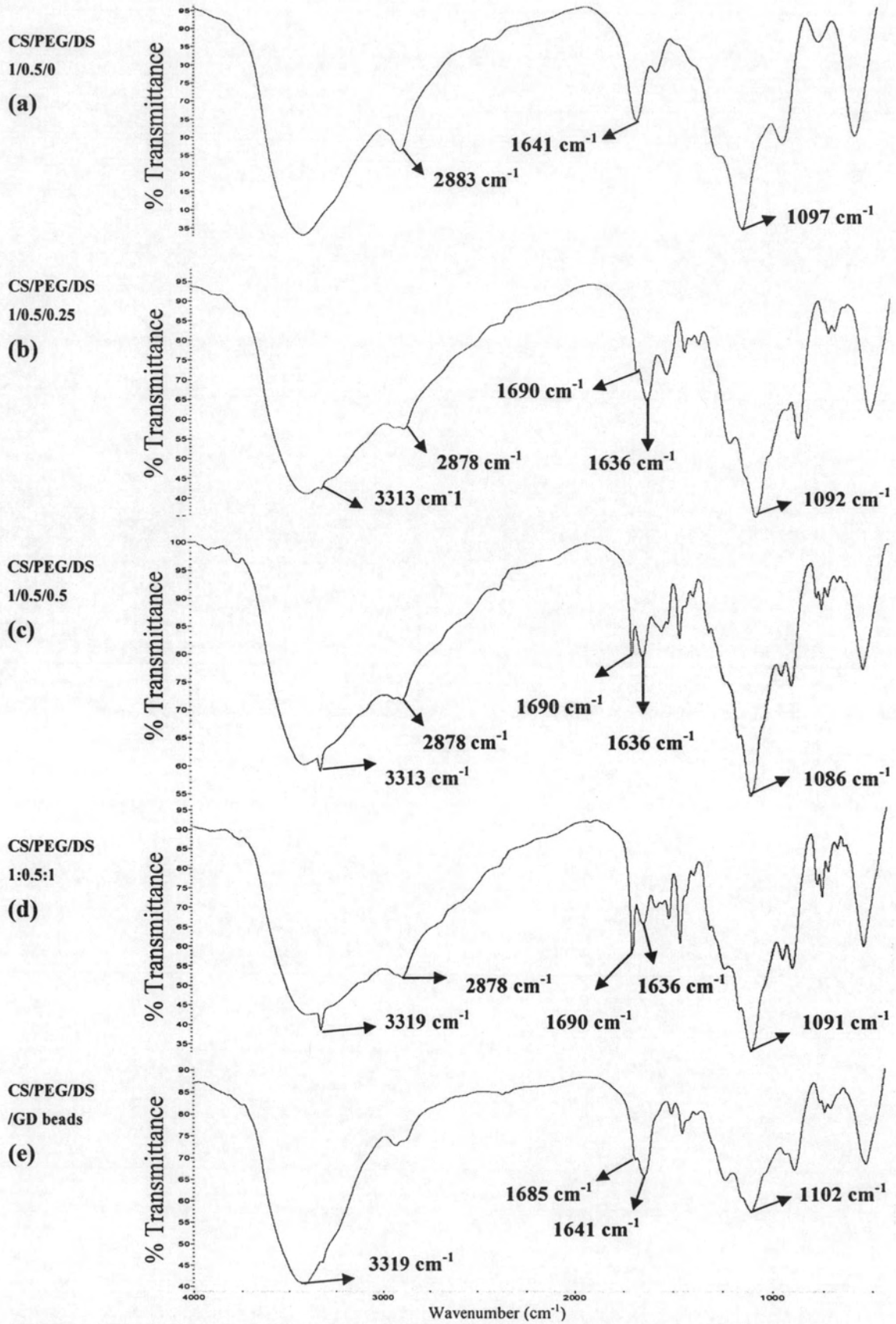


Figure 4.13 FT-IR spectrum of (a)-(d) CS/PEG/DS beads were varied in polymer to drug ratios; (a) 1:0.5:0, (b) 1:0.5:0.25, (c) 1:0.5:0.5, (d) 1:0.5:1 (e) CS/PEG/DS/GD beads

4.2.4 Differential scanning calorimeter (DSC)

The DSC thermograms of the beads obtained from various compositions are shown in Figure.4.14 (a)-(h).

Comparison of the DSC thermograms of the pure chitosan, the pure TPP and the chitosan/TPP beads are shown in Figure.4.14 (a)-(c). It was revealed the endothermic broad peak at 93.2°C and exothermic peak at about 300°C in the pure chitosan bead. The two peaks represent the evaporation of water and the degradation of chitosan, respectively. The TPP thermogram showed an endothermic peak at 121.6°C , it was indicated that there is an evaporation of water similar to that from the pure chitosan bead (Figure 4.14 (b)). The DSC thermogram of bead which was obtained from the mixing of pure chitosan and TPP show in Figure.4.14(c). The first endothermic occurred at 79.7°C. It is lower than that which were obtained from pure chitosan (93.2°C) and TPP (121.6°C). The second endothermic occurred at higher temperature (200.2°C). Because of the strong ionic interaction between cationic polymer chitosan and highly charge anionic TPP, the high temperature is need to break their interaction.

Figure.4.14 (d) reveals the DSC thermogram of pure DS. There are three endothermics peaks. The first one is a sharp endothermic peak at 52.8°C and the second one at 100.2°C. The first two endothermic peaks showed the water loss from the bead. The third endothermic peak of DS at 288°C. It is the melting point of DS. Moreover, there is an exothermic peak at about 300°C because the fusion of the solvated crystals and oxidation reaction between DS and oxygen in air environment fusion.

The comparison between the DSC thermogram of chitosan/TPP (Figure.4.14(c)) and DS-loaded chitosan/TPP bead (Figure.4.14(e)), the DSC thermogram of DS-loaded chitosan/TPP bead shows the combination of polymer and drug thermogram. The characteristic peak of chitosan/TPP and DS are still presented but with the slightly shifted from the initial positions. Although DSC thermogram of DS-loaded chitosan/TPP have the same characteristic peak of chitosan/TPP and DS, the different DSC thermogram of chitosan/TPP and DS-loaded chitosan/TPP bead

was significantly. The endothermic peak at 200.2°C was not observed thermogram of DS-loaded chitosan/TPP but there is a new endothermic peak at 173.2 °C. The results indicate that the chemical interaction between DS and the composition inside the bead was very strong.

Finally, the thermal behavior of the unloaded-DS chitosan/TPP/PEG and loaded-DS chitosan/TPP/PEG were shown in the DSC thermogram Figure.4.14(g) - (h), respectively. The DSC thermogram of the unloaded-DS chitosan/TPP/PEG bead show only one endothermic peak at 75.6°C. It was not observed the characteristic peaks of chitosan/TPP and DS. The DSC thermogram of the loaded - DS chitosan/ TPP/PEG bead present 4 endothermic peaks at 54.9°C, 73.8°C, 192.4°C and 210.6°C. The water was losen from the bead at 54.9°C and 73.8°C. At 192.4°C, the interaction between DS and chitosan/TPP/PEG was broken. When it was heated to 210.6°C, chitosan was segregated from TPP/PEG.

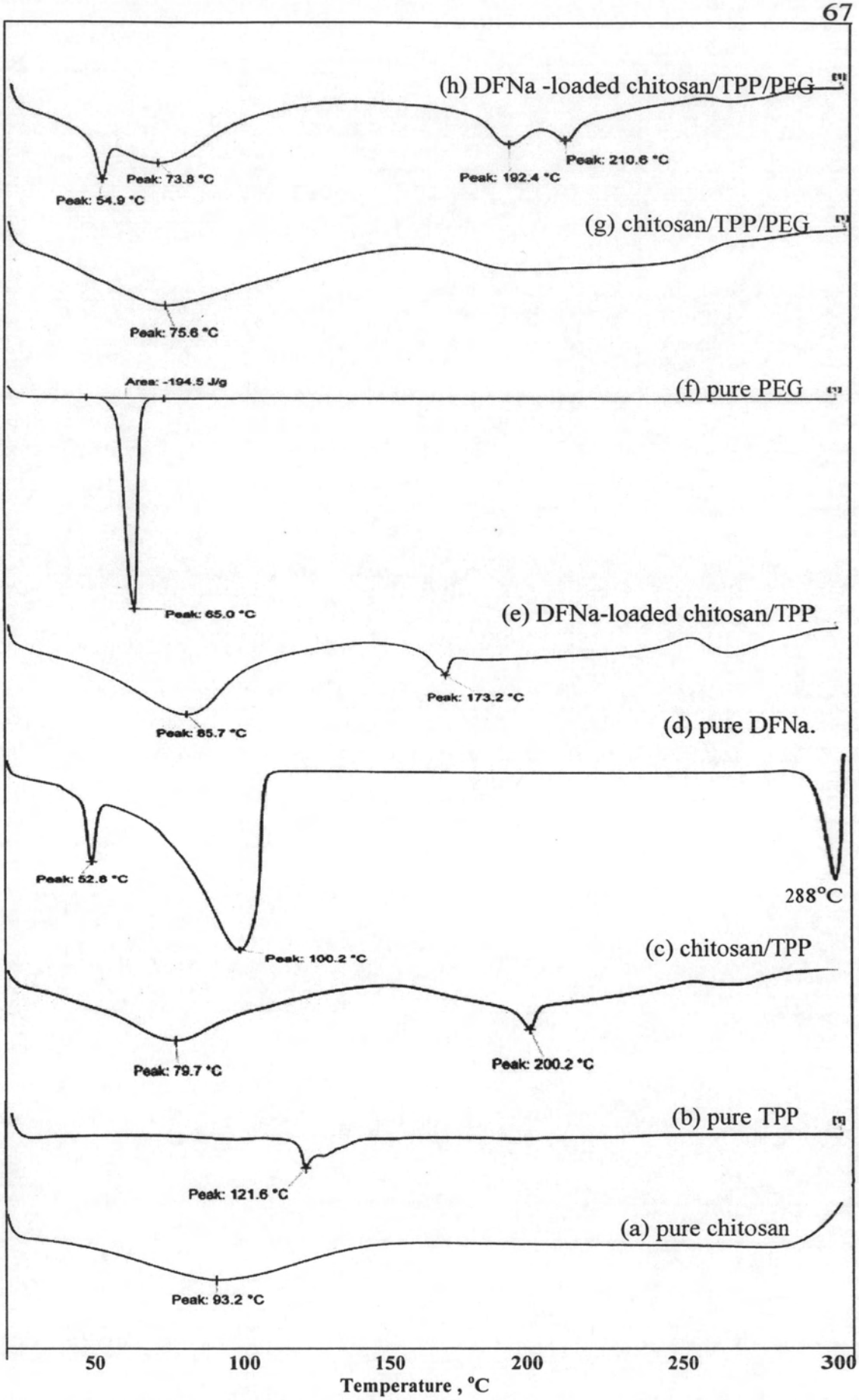


Figure 4.14 The DSC thermograms of the beads obtained from various compositions

4.3 The Encapsulation Efficiency of the Beads

The percentages of encapsulation efficiency (%EE) of the DS-loaded chitosan beads prepared from various compositions are shown in Table 4.4-4.5.

Effect of a cross-linking agent

The chitosan beads were prepared in the different coagulants should give different %EE. All of formulations of chitosan beads prepared with tripolyphosphate gave higher %EE (Formulation C to H, 58.16-95.41%) than that of the chitosan beads prepared with 5% NaOH (Formulation A1, 55.79 %). In addition, the morphologies of cross-section network of Formulations A1-B from Figure 4.1 were consistency to results of %EE. Formulation A1 had the large porous network inside the beads; therefore, it was unable to entrap a large amount of the drug within the chitosan network. In contrast, polyelectrolyte complex network formed by ionic interactions of $P_3O_{10}^{5-}$ ions of TPP presented the dense network with small porous. Thus the drug was preferably entrapped into the chitosan-TPP network and obtained the high %EE.

Effect of chitosan to DS ratio

The chitosan to DS ratios were varied in formulations C to H and the values of the %EE from these beads were also different. The %EE of Formulation C to H was in the range of 58% to 95%, depending on the composition in the formulations. In addition, the decrease of %EE of the beads were found when DS content were increase in Formulation C to H. Figure 4.4, Formulation C gave the highest percentage of %EE at 95.41% while the lowest percentage of %EE was found in Formulation H at 58.16%. From a result of the 1/2 proportion of chitosan/DS in Formulation H, it was too much amount of drug for completely entrapped in chitosan matrix. In contrast, the high %EE of Formulations C was comparable to that of Formulation D, because the low proportion of DS made it possible to entrapped most of the drug. Furthermore, the most suitable chitosan/DS ratio would be considered together with the drug releasing results from Figure 4.25. Formulation D presented the slower drug release profile. Hence, the suitable ratio of chitosan to DS for improving drug entrapment and controlled release character was chitosan /DS (1/0.5) in formulation D.

Table 4.5 The encapsulation efficiency (%EE) of the formulated chitosan beads with various compositions.

Formulation	ratio of compositions			Tripolyphosphate		Time (min.)	% EE ± S.D.
	chitosan	PEG	DS	conc. (%w/v)	pH value		
A0	1	0	0	-	-	20	-
A1	1	0	1	-	-	20	55.79±0.46
B	1	0	0	1	6.0	20	-
C	1	0	0.25	1	6.0	20	95.41±3.89
D	1	0	0.5	1	6.0	20	91.98±1.89
E	1	0	0.75	1	6.0	20	83.22±3.89
F	1	0	1	1	6.0	20	87.37±4.34
G	1	0	1.5	1	6.0	20	81.06±3.47
H	1	0	2	1	6.0	20	58.16±3.55
I	1	0	3	1	6.0	20	-
D	1	0	0.5	1	6.0	20	91.98±1.89
J	1	0	0.5	5	6.0	20	95.00±2.68
K	1	0	0.5	10	6.0	20	90.45±4.48
K	1	0	0.5	10	6.0	20	90.45±4.48
L	1	0	0.5	10	3.0	20	90.03±2.79
M	1	0	0.5	10	8.0	20	58.02±3.48
K	1	0	0.5	10	6.0	20	90.45±4.48
N	1	0	0.5	10	6.0	30	93.34±0.62
O	1	0	0.5	10	6.0	60	88.95±2.13
N	1	0	0.5	10	6.0	30	93.34±0.62
P	1	0	0.5	10	6.0	30	93.39±0.75
Q	1	0	0.5	10	6.0	30	82.87±0.40

The %EE could not be determined because the beads were not successfully prepared.

[b] The bead without DS

[*] The beads formed by using a needle number 22.

[**] The beads formed by using a needle number 24.

Effect of TPP conditions

The TPP coagulant would create an electrostatic interaction with chitosan, so TPP condition should be important for forming firmed beads. From table 4.4, concentrations of TPP for chitosan-TPP bead preparation was increased to study the various proportion of entrapment efficiency. After beads forming by the various concentrations, %EE of each formulation were different. The various concentration of TPP at 1%, 5% and 10% gave high %EE in 91.98%, 95.00% and 90.45%, respectively. Due to formulation J prepared by 5% TPP could give high amount of TPP anions for polyelectrolyte complex forming with chitosan. Therefore DS could be firmly entrapped inside chitosan-TPP beads and it possible to present the high percentage of drug entrapment. In contrast to Formulation K, 10% TPP using as a coagulant gave the lowest percentage of encapsulations, because the very dense network inside beads, formed by high amount of TPP, made drug difficultly diffused into beads, so %EE of Formulation K was lower than others. Although %EE of Formulation K was quite low but when considered along with the released behavior, presented on Table C1 (Appendix C), Formulation K could release almost entrapped drug up to 97.08% within 24 hours. Unlike, Formulation D and J could release drug only at 50.72 and 76.40% which was unacceptable for the pharmaceutical application. To gain the behavior in drug release, the suitable formulation should be prepared at 10% of TPP which is Formulation K.

The concentrations of TPP coagulant were varied in previous study, so this part should be studied in various pH conditions of TPP (pH 3.0, 6.0 and 8.0). Because the charge number of chitosan and TPP were changed at various pH conditions, so to find the most suitable condition of TPP, it should be considered at each circumstance. At acid condition, pH 3.0, the dense network inside beads, prepared by electrostatic interaction of TPP ions, could entrapped the high amount of DS which resulted in high drug entrapment efficiency at 90.03% of Formulation L. Likewise, further increase of solution to pH 6.0 (Formulation K), chitosan beads also displayed the high percentage of drug entrapment efficiency at 90.45% [37].

But when the pH value was changed to over pH 8.0, the ionization degree was decreased dramatically. The polyelectrolyte complex between chitosan and TPP became the loosen network. Because of this, Formulation M, formed at TPP solution pH 8.0, gave the poor entrapment efficiency at 58.02%.

In pharmaceutical field the moderate pH condition are preferred therefore Formulation K, prepared at pH 6.0, should be the better condition for further studies.

Effect of cross linking time

As the results of the suitable condition of TPP coagulant was 10%(w/v) at pH 6.0 which the gelation of chitosan beads was controlled by ionic crosslinking throughout the beads. At pH 6.0, TPP presented $P_3O_{10}^{5-}$ and a few of OH^-, so the strong networks of chitosan beads should be depended on the diffusion of $P_3O_{10}^{5-}$ ions, in other words it depended on cross-linking time.

The cross-linking times were studied in 20, 30 and 60 minutes, resulted at Table 4.4 in name Formulation K, N and O, respectively. The chitosan beads Formulation N gave the highest %EE in 93.34%, whereas the Formulation K and O presented lower efficiency at 90.45% and 88.95%, respectively. Because of the too long of the cross-linking time, more DS could diffuse throughout of beads, so lower drug entrapment was presented in Formulation O.

On the other hand, from the photomicrographs in Figure 4.5 the network inside beads should be formed completely at 20-30 minutes of cross-linking time. Both Formulation K and N presented the high drug efficiency; especially because the denser and stronger network inside Formulation N made it showed the highest %EE. Therefore, the greatest cross-linking time of chitosan-TPP beads was 30 minutes.

Effect of chitosan bead size

The Formulation N, P and Q from Table 4.4 were formulations of chitosan beads prepared by the different sizes of syringe needles. As the same of chitosan hydrogel condition, %EE of those three formulations would not be different. Although the same condition of chitosan hydrogel and TPP solution, beads from Formulation Q gave the lowest percentage at 82.87%. Because of the high viscosity of chitosan hydrogel made the difficult dropping through the syringe needles number 24, so the high beads sizes distributions and low %EE were happened. For this reason the bigger sizes of the syringe needles, number 18 and 22 could make the easier dropping of chitosan beads and the high drug entrapment efficiency would be achieved. Therefore the most suitable formulation of chitosan beads sizes were considered together with the release of drug at Table C1 (Appendix C).

Table 4.5 The encapsulation efficiency (%EE) of the formulated chitosan/PEG beads with various compositions.

Formulation	ratio of compositions			Tripolyphosphate		Time	% EE ± S.D.[a]
	chitosan	PEG	DS	conc. (%w/v)	pH value	(min.)	
P	1	0	0.5	10	-	30	93.39±0.76
PEG0	1	1	0	10	6.0	30	-[b]
PEG3	1	1	0.5	10	6.0	30	90.59±2.00
PEG1	1	0.25	0.5	10	6.0	30	98.81±1.97
PEG2	1	0.5	0.5	10	6.0	30	92.10±3.20
PEG3	1	1	0.5	10	6.0	30	90.59±2.00
PEG4	1	2	0.5	10	6.0	30	98.88±2.47
PEG5	1	0.5	0.25	10	6.0	30	95.70±0.36
PEG2	1	0.5	0.5	10	6.0	30	92.10±3.20
PEG6	1	0.5	1	10	6.0	30	90.71±2.41
PEG7	1	0.5	1.5	10	6.0	30	95.00±2.68

[b] The bead without DS

The effect of chitosan/PEG ratio

The optimum condition of chitosan/TPP beads was obtained in the previous studies. PEG was another polymer that used for improved the release profiles of chitosan beads to slower behavior.

From Table 4.5, %EE of chitosan/PEG beads were compared with those of chitosan/TPP beads. The results of %EE of chitosan, reinforced by PEG, Formulation PEG1-PEG4, did not show a significantly different efficiency.

In addition, the drug content on chitosan/PEG beads were increased, the drug entrapment efficiency were give the insignificant difference. Therefore, the suitable condition of chitosan/PEG ratio was concerned by the results of %drug release from Table C1 (Appendix C).

4.4 Swelling Analysis

Swelling Analysis in pH 1.2 (Stimulated Gastric Fluid, SGF)

The pK_a of chitosan is around 6.3. Thus, $-NH_2$ sites on CS will be protonated to $-NH_3^+$ when surrounded in the acidic medium. A large number of $-NH_3^+$ could generate the ionic repulsion and resulted in swelling behavior. In addition, there are many factors that affect for considering on the swelling behavior of CS beads [13].

The amount of DS effects

In general, DS is the cationic compound that presents COO^- group. Therefore, the interaction between COO^- and NH_3^+ of chitosan could possibly occur. The swelling ratio of the CS loaded DS beads were shown Figure 4.15 and Table B1 (Appendix B).

The results showed that the swelling ratio of CS loaded DS beads decreased when increased the drug amount. This is possibly due to the higher amount of drug which consists of more COO^- groups, exhibit the greater interact with $-NH_3^+$ groups of chitosan. Therefore, less ionic repulsion occurring between NH_3^+ groups themselves which reduce the result in swelling ratio.

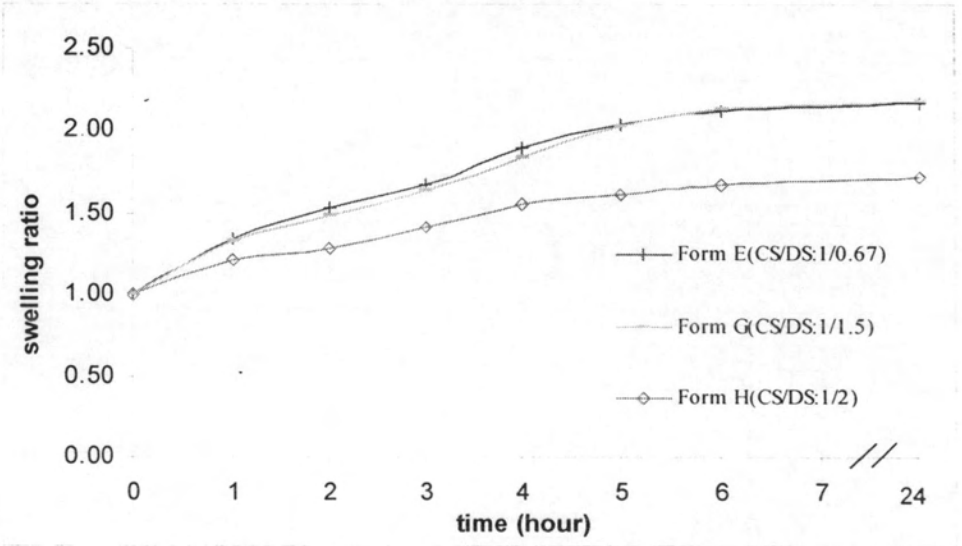


Figure 4.15. Swelling behavior of various ratios of chitosan/sodium diclofenac beads in SGF.

Effect in the concentrations of TPP coagulant

The multivalent anionic of TPP could directly interact with NH_3^+ groups on CS. Figure 4.16 and Table B1 (Appendix B) presented the swelling ratios of chitosan beads prepared from various conditions of TPP.

As the results, higher percentage of TPP contributed to the higher strength of network with chitosan by the ionic interaction between multivalent anionic of TPP and NH_3^+ sites, therefore the less swelling ratio of chitosan beads was observed by increasing the concentration of TPP.

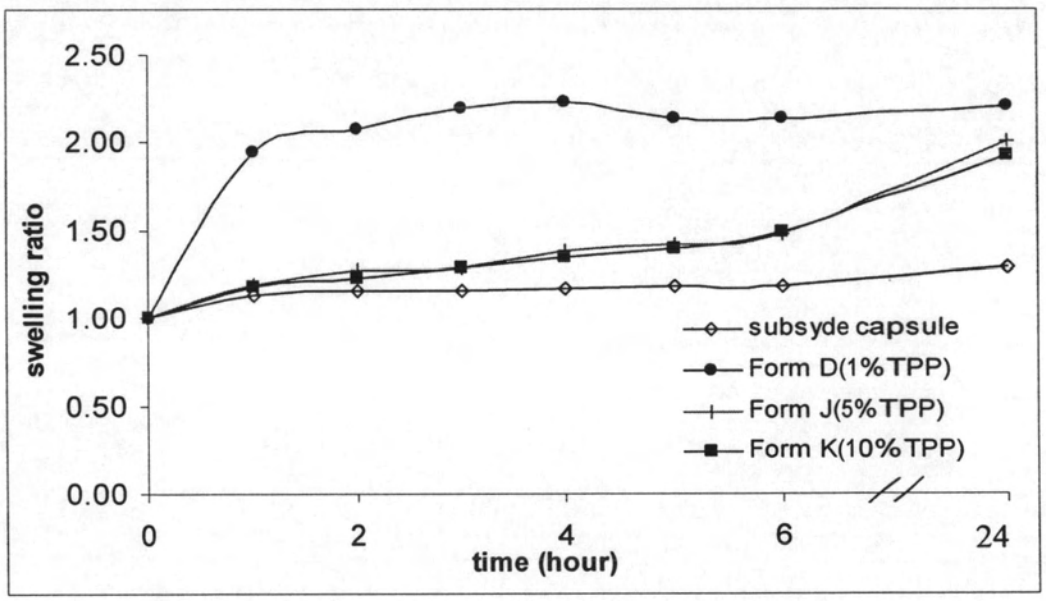


Figure 4.16 Swelling behavior of various concentration of TPP solution in SGF.

Effect in the proportions of chitosan to PEG

Another parameter to be considered is the amount of blending polymer, PEG. The results of the swelling ratio of CS beads enhanced with and without PEG were displayed in Figure 4.17 and Table B1 (Appendix B).

Incorporation of PEG in the chitosan beads exhibited the higher swelling properties. In addition, the erosion of CS/PEG beads was observed after 6 hours. This is probably due to the PEG could be swellable in acidic medium results in enhancing the swelling of chitosan beads. The proportions between CS/PEG are not shown significantly different results which were illustrated in Figure 4.17.

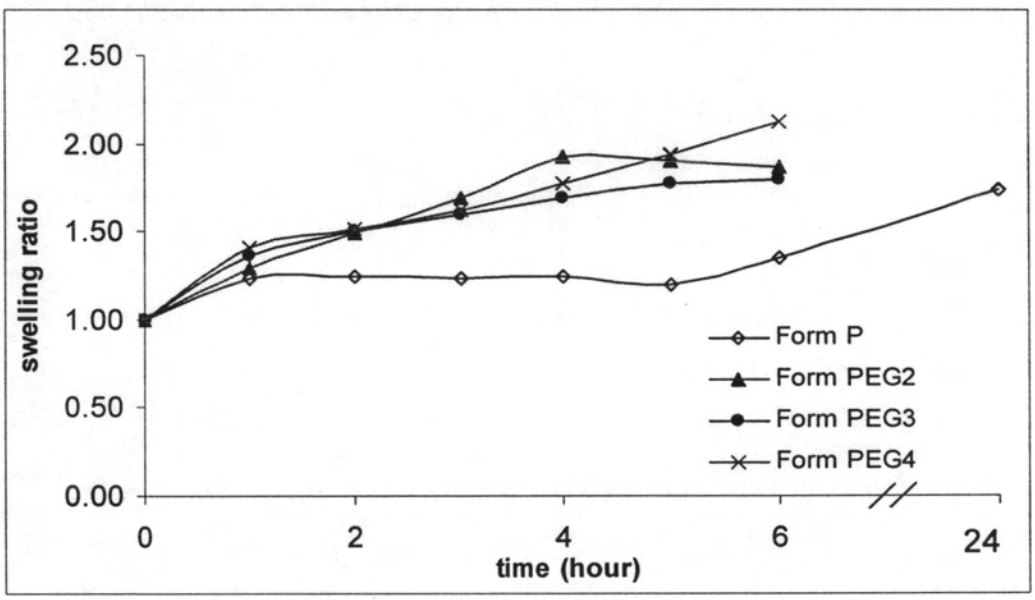


Figure 4.17 Swelling behavior of various ratios of chitosan/PEG beads in SGF.

Swelling Analysis in pH 7.4 (Stimulated Intestinal Fluid, SIF)

At the pH of solution over the pK_a value of chitosan that used as the main composition in each formulation, amino groups are less ionized [30] that results the swelling properties of chitosan are then lower. Proposed that less than 10% of amino groups of chitosan were ionized in the basic medium with pH over 7.5 [35].

The swelling ratios of the beads with various compositions in SIF pH 7.4 are shown in Figure 4.18-4.20 and (Table B2, Appendix B). The swelling behaviors of the beads for all formulations in this dissolution system (phosphate buffer saline pH 7.4) were not significantly different. This is due to the chitosan and polyethylene glycol showed less swellable behavior in the basic medium.

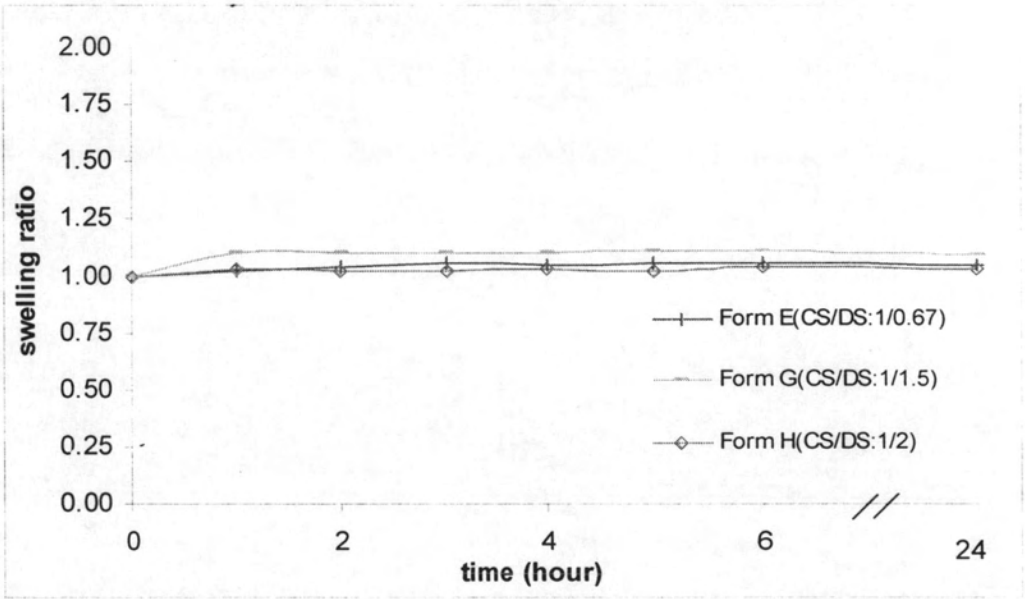


Figure 4.18 Swelling behavior of various ratios of chitosan/sodium diclofenac beads in SIF.

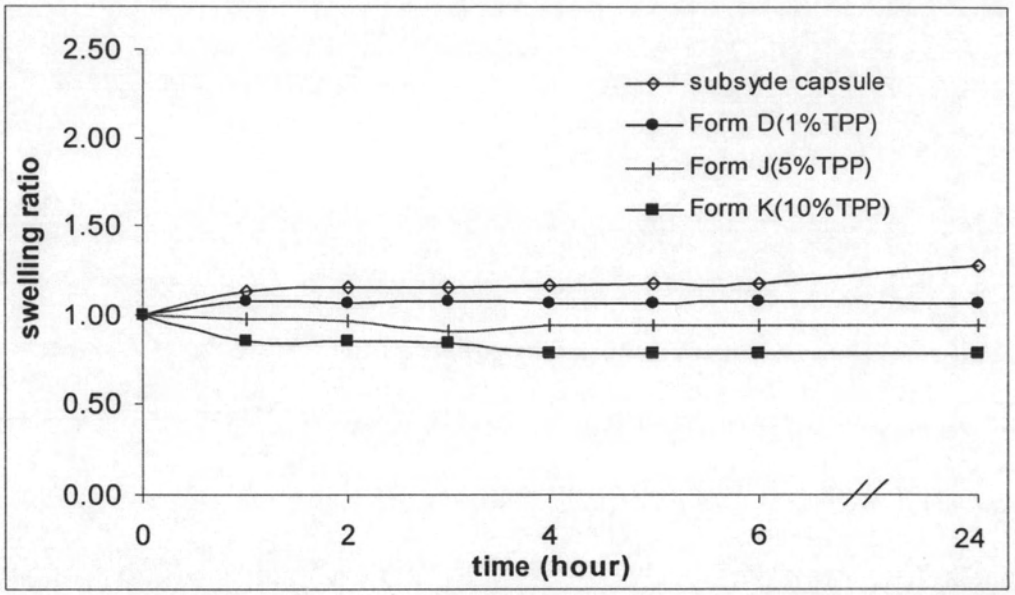


Figure 4.19 Swelling behavior of various concentration of TPP solution in SIF.

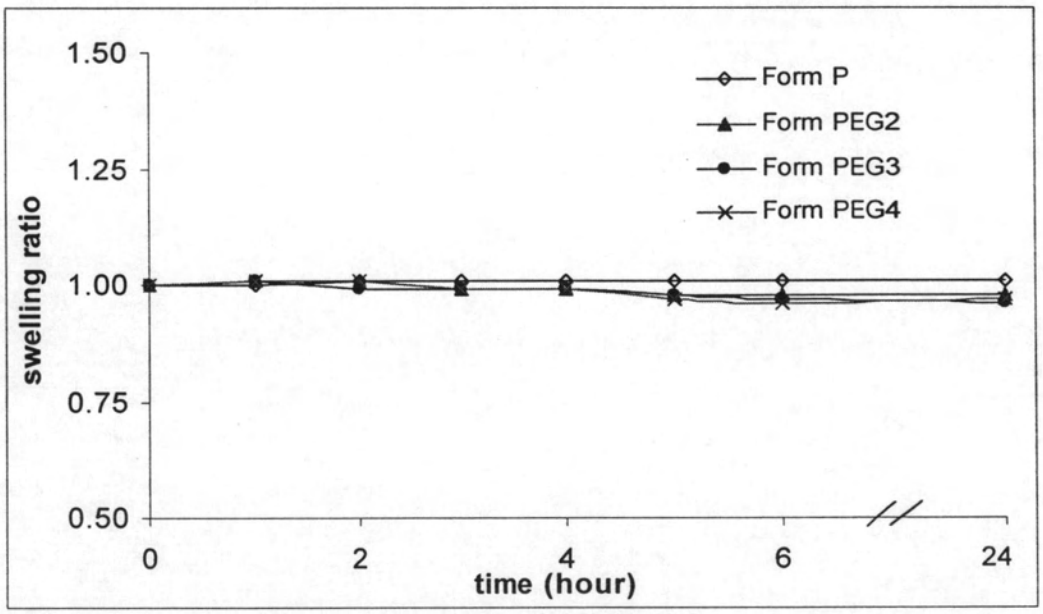


Figure 4.20 Swelling behavior of various ratios of chitosan/PEG beads in SGF.

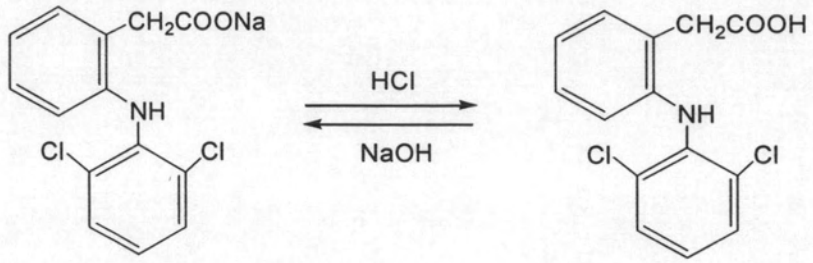
4.5 In Vitro Release Study

4.5.1 Dissolution profiles and release rate profiles

The dissolution profiles of DS can be described as graphs to explain and understand the drug released behavior from the beads. The dissolution and release rate data of each formulation are given in Table C1 (Appendix C).

The release rate was calculated by dividing the difference of percent drug release at various time intervals with the time utilized to release that certain amount of drug.

The release studies of DS from the commercial products and Formulation A to GLD7.5 (see Table 3.2, Chapter 3) were evaluated in 0.1N HCl (pH 1.2), phosphate buffer saline pH 6.6, and pH 7.4 by the pH-alternating method. In the pH-alternating system, the beads were tested in acidic condition (0.1N HCl, pH 1.2) for 2 hours. At the third hour, the dissolution medium was changed to the phosphate buffer saline pH 6.6 for 1 hour. Then, the beads were subjected to the phosphate buffer saline pH 7.4 and were continuously carried out up to 24 hours. These dissolution profiles and release rate profiles are shown in Figure 4.22 and 4.29. In an acidic condition, the release rate was slow because diclofenac sodium became diclofenac acid which is an insoluble form. Therefore, it is not dissolved out from the beads. When the pH of a dissolution medium is higher, the drug was rapidly released from all formulations because an insoluble form of diclofenac acid was completely converted to a soluble form again [33, 34].



Sodium diclofenca Diclofenac acid

Figure 4.21 The acid-base reaction of sodium diclofenac and diclofenac acid.

4.5.1.1 *The dissolution profiles of the commercial diclofenac sodium product*

Subsyde® CR capsules (as 100 mg).

Approximately 1/2 of the capsule from the Subsyde® CR capsule (about 50 mg) were evaluated in the pH-alternating system. The amounts of drug release at specific time intervals and release rate data are presented in Table C1 (Appendix C). The dissolution profile and the release rate profile of DS from the Subsyde® CR capsule were affected by the release dissolution medium as illustrated in Figure 4.22 and 4.23. In the pH-alternating system, the drug release from the Subsyde® CR capsule was less than 1% in the first 2 hours (pH 1.2) and was less than 16 % in the phosphate buffer saline pH 6.6. The drug release was more than 90% within the sixth hour in the phosphate buffer saline pH 7.4. After 24 hours, the percentage of drug released was increased to 94.97% while the beads were still in the original shape and with no erosion.

The Voltaren® tablet (as 25 mg).

The percentages of drug release and data of the dissolution test of Voltaren tablet drug were shown in Table C1 (Appendix C). The dissolution profiles of DS from this tablet gave the low percentage at the first 3 hours, at less than 1%. After the dissolution medium was changed to phosphate buffer pH 7.4, it was found that this Formulation gave the burst release of DS.

The Voltaren® SR tablet (as 100 mg).

The percentages of drug release and data of the dissolution test of Voltaren SR tablet drug were shown in Table C1 (Appendix C). This Voltaren SR tablet gave the good result in drug release. It presented a low release profile since the beginning state and kept slow released behavior from time to time until 24 hours. In detail, it gave only 10% of DS release at first 4 hours while 45% of DS was released at 6 hours later. The maximum dissolution tested at 24 hours was about 85%.

The dissolution profiles and release rate profiles of the commercial DS products were compared in Figure 4.22-4.23. As the results, it has been noticed that most of the commercial DS products tested gave the wide range in the drug release time.

4.5.1.2 *The dissolution profiles of the chitosan containing with and without PEG beads : formulation A-PEG7*

Effect of types of coagulant solution

The dissolution profiles of DS from the beads with two coagulants; namely 5% NaOH (Formulation A1) and 1% TPP (Formulation F) (see Table 3.2 Chapter 3) in 0.1N HCl, phosphate buffer pH 6.6 and pH 7.4 using the pH-alternating method are shown in Figure 4.22 (Table C1, Appendix C). Each point represents the average value obtained from three determinations at each sampling time.

In the pH-alternating system, the averages drug release from the chitosan beads prepared through 5% NaOH (Formulation A1) and 1% TPP (Formulation F) were different. Formulation F gave the higher performance in delayed release in HCl pH 1.2 (lower than 3%) and slightly increased in pH 6.6 (up to 3.65%). After the beads were moved to the release dissolution medium pH 7.4, the DS was rapidly released at the initial time. After that the rate of the drug release was controlled. The DS release of Formulation F over 24 hours was 32.80%.

Although the final percentage of drug release at 24 hours of Formulation A and F was similar (34.93% and 32.80%) but the dissolution profiles was different. During the 2^{nd} to 3^{rd} hour, the drug release of 33.55% from Formulation A1 is significant higher than that of 15.21% from Formulation F. This is probably due to the effect of the type of crosslinking agent on the swelling behavior of the beads. In the medium pH 6, when the beads contained only chitosan, which is a polycationic and presents $-NH_3^+$ sites, leading to the ionic repulsions within the beads and causing the beads swelled. Therefore the drug was easily diffused out. For Formulation F which consisted of chitosan and TPP, chitosan presents $-NH_3^+$ sites and TPP dissociates to give phosphoric ions in acid. The interaction between the cations in chitosan and the anions in TPP made them less swelling than the Formulation A1. There is a limited swelling of the beads, which prevent the drug release.

In addition the TPP could form the stronger ionic cross-linking networks inside beads as can be seen from the SEM photomicrographs in Figure 4.1. Therefore, TPP solution should be the suitable coagulant in chitosan preparation for drug delivery system.

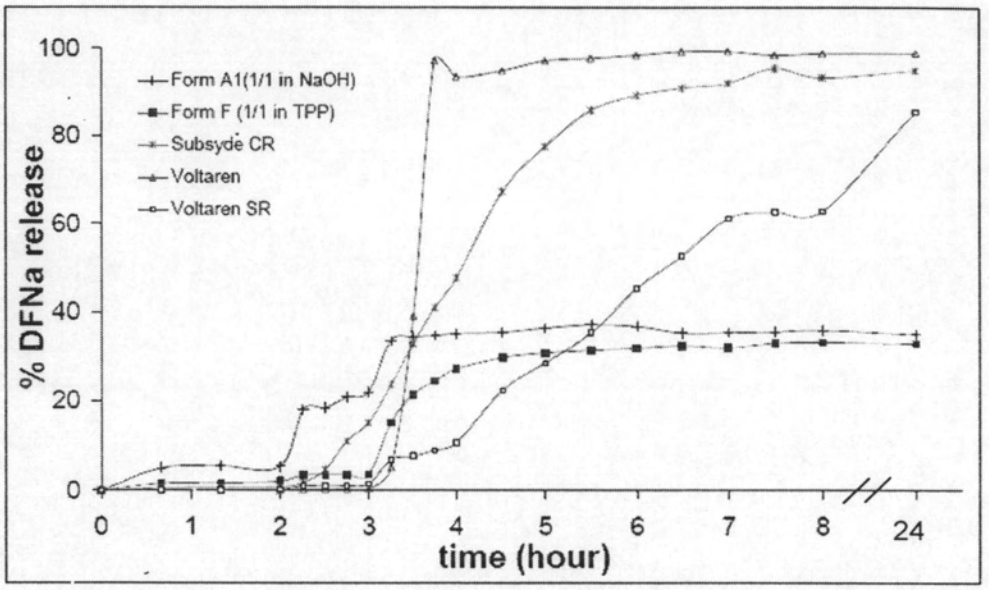


Figure 4.22 The dissolution profiles of sodium diclofenac from the beads with various coagulant in the pH-alternating system; A1: NaOH, F: 1%TPP.

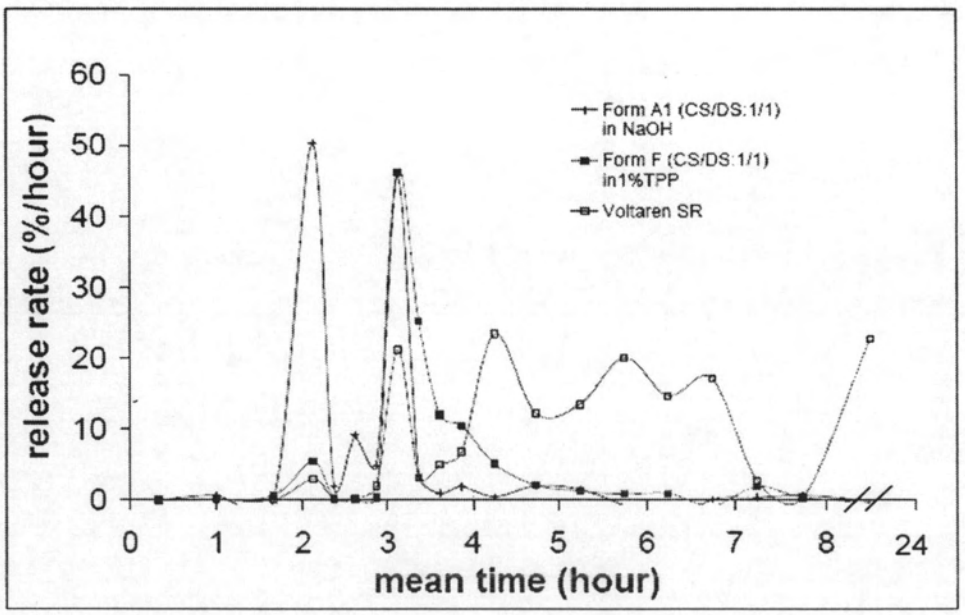


Figure 4.23 The dissolution profiles of sodium diclofenac from the beads with various coagulant in the pH-alternating system; A1: NaOH, F:1%TPP.

Effect of chitosan to DS ratios on the dissolution profiles

The DS content in chitosan hydrogel was important not only for %EE but also for the releasing profiles. Thus the various chitosan to DS ratios were studied. The ratios were varied from 1/0.33 to 1/3 of chitosan to DS, Formulation C to H are presented in Table 3.2, Chapter 3

In the pH-alternating system, at pH 1.2, the drug release from Subsyde® CR tablet was negligible (less than 1%). Meanwhile, the drug release from Formulation C to H were approximately 3-5% because the beads were partially swelled, leading to the drug was eluted. Then, the dissolution medium was changed to the phosphate buffer saline pH 6.6 for 1 hour. The drug still dissolved into the dissolution medium pH 6.6 up to 3-4%.

When the dissolution medium was changed to the phosphate buffer pH 7.4, the release profiles of all studied Formulation are similar. The drug is rapidly released at the first time interval. After the fourth hour, the release rate is slow. Formulation D, 1/0.5 chitosan/DS, give the highest percentage release of DS (50.72%) over 24 hours. Whereas Formulation E (1/1 chitosan/DS) releases the lowest amount of drug only at 32.80%. From this results showed that the drug was lost during they were in the acidic medium. This is because the 1%TPP may not be the most suitable condition for forming the strong cross-linking networks inside beads, therefore the variations of the condition of TPP coagulant were studied.

In this section, the Formulation D give the highest released drug over 24 hours, therefore it is the most suitable ratio of chitosan/drug for using in the further experiment.

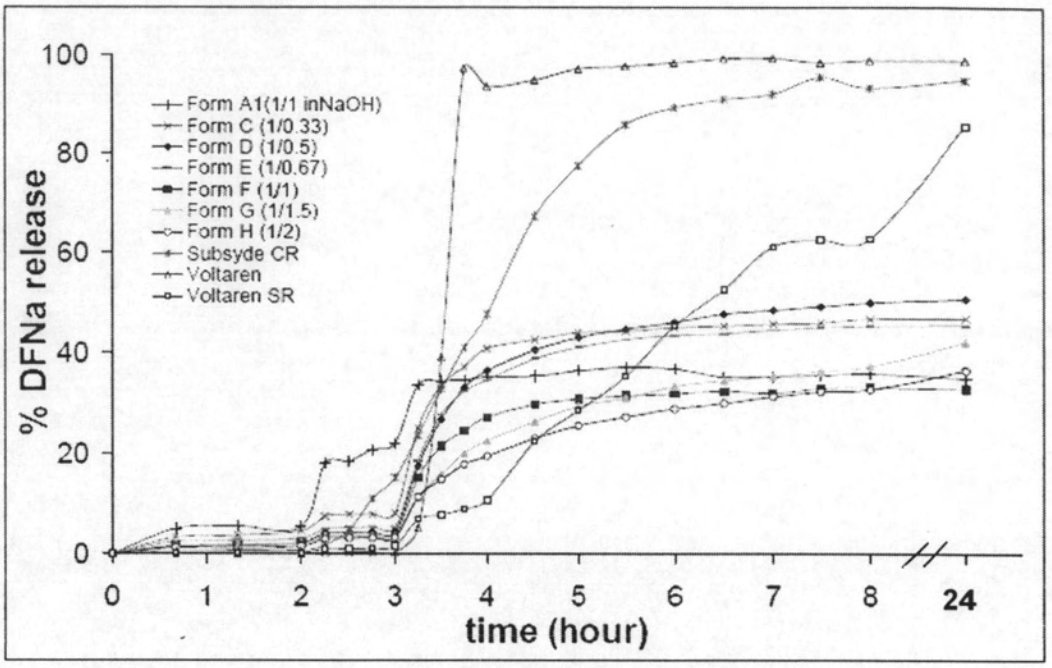


Figure 4.24 The dissolution profiles of sodium diclofenac from the beads with various chitosan/DS (CS/DS) ratios in the pH-alternating system.

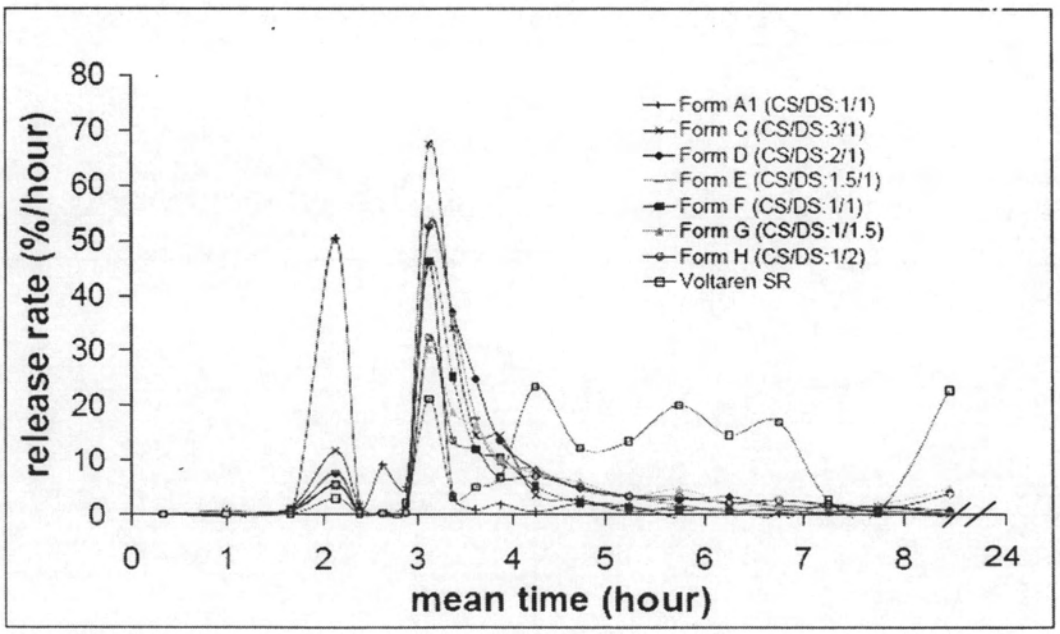


Figure 4.25 The release rate profiles of sodium diclofenac from the beads with various chitosan/DS (CS/DS) ratios in the pH-alternating system.

Effect of TPP coagulant concentration on the dissolution profiles

From the previous study on the effect of chitosan/DS ratios on dissolution profiles, it was found that 1%TPP was not the suitable condition of a coagulant for forming the beads. To determine the suitable condition of a coagulant, the various concentrations of TPP were studied at 1%, 5% and 10% (w/v), namely Formulation D, J and K, respectively (see Table 3.2, Chapter 3). The dissolution profiles and release rate profiles of DS in various concentrations of TPP are given in Figure 4.26-4.27.

When using 1%TPP as a crosslinking agent (Formulation D), the total drug released was only 50.72%, indicating that a high amount of diclofenac sodium transformed into an precipitated form of diclofenac acid in pH 1.2 and pH 6, which cannot be detected by UV technique. That was because the polymer network is not strongly formed as can be seen from the large porous network in the photomicrograph in Figure 4.3.

When using 5% and 10% TPP as a crosslinking agent (Formulation J and K respectively), their patterns of drug release were similar except that the total drug released of Formulation K was higher at 97.08%, compared to 76.40% of Formulation J. The higher percentage of TPP contributed to the stronger network and consequently less drug loss in the first three hours at an acidic condition [38].

Therefore, Formulation K was the most suitable formulation. These dissolution results are consistent with the density of the polymer network as shown in Figure 4.3

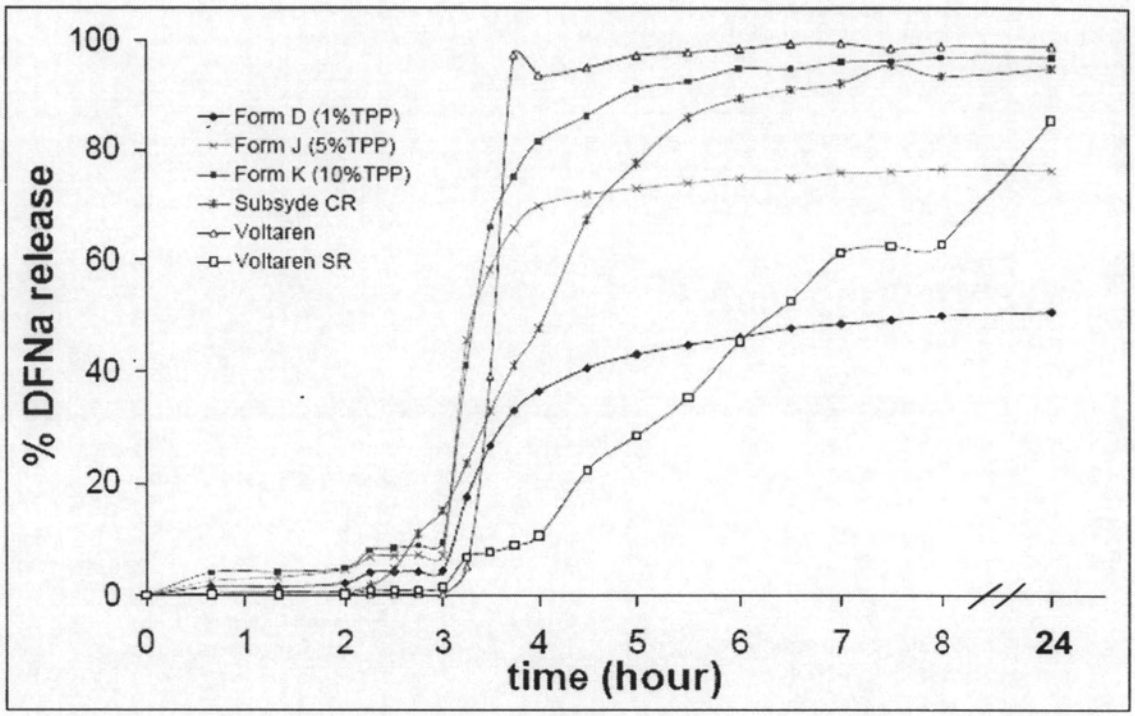


Figure 4.26 The dissolution profiles of sodium diclofenac from the beads with various coagulant concentration in the pH-alternating system.

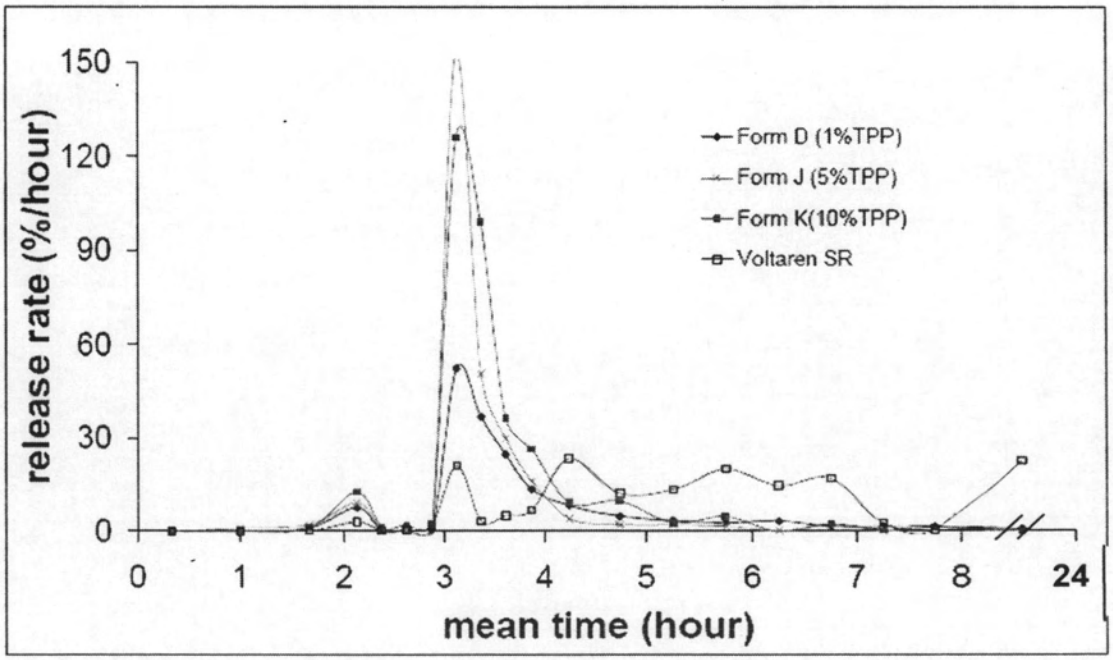


Figure 4.27 The release rate profiles of sodium diclofenac from the beads with various coagulant concentrations in the pH-alternating system.

Effect of the pH value of TPP coagulant on the dissolution profiles

Chitosan with a pK_a of 6.3 is polycationic when dissolved in acid and presents $-NH_3^+$ sites. Sodium tripolyphosphate ($Na_5P_3O_{10}$) dissolved in water dissociates to give both hydroxyl and phosphoric ions. Since the cross-linking of chitosan would be dependant on the availability of the cationic sites and the negatively charged species, it was expected that the pH of TPP would play a significant role. pH would bring about a change on the extent and type of cross-linking. Hence in the present study, 2 different pH conditions namely pH 3 and pH 9 were used for obtaining the cross-linked particles. When the pH of TPP was adjusted to 3, only phosphoric ions were present. However, at pH 9, both OH^- and phosphoric ions were present and may compete with each other to interact with the $-NH_3^+$ of chitosan [36].

In general, the charge number anions and chitosan are all mainly controlled by solution pH. Thus, the pH value of TPP coagulant was another important factor in chitosan beads forming and in controlled drug release.

It was observed that the best formulation which gave a good controlled release profiles can be found by varying pH condition of TPP. The dissolution profiles and the release rate profiles are available in Figures 4.28-4.29.

In the pH-alternating system, Formulation L, K and M were prepared by using 10%TPP at pH 3.0, 6.0 and 8.0, respectively. The percentage of DS release was presented in Table C1 (Appendix C).

At the first three hours of the dissolution test, each formulation presented the slightly increasing release profiles with less than 10% in the third hour. After that, the solutions were changed to the final system, pH 7.4. The release profiles of each formulation were slightly different. All Formulations gave the high percentage of drug release over 80% at the 4^{th} hour and after that the drug release was slightly increased until the 24^{th} hour.

The release rate of Formulation M (pH 8.0) is shown that it is a sustained release formulation; however, the percent encapsulation is only 58.02%. Therefore this formulation is not a good formulation.

Both Formulation K and L had the highest drug release and encapsulation efficiency. However, Formulation K was selected for subsequent study because the coagulant condition is nearly neutral which is more acceptable for pharmaceutical applications.

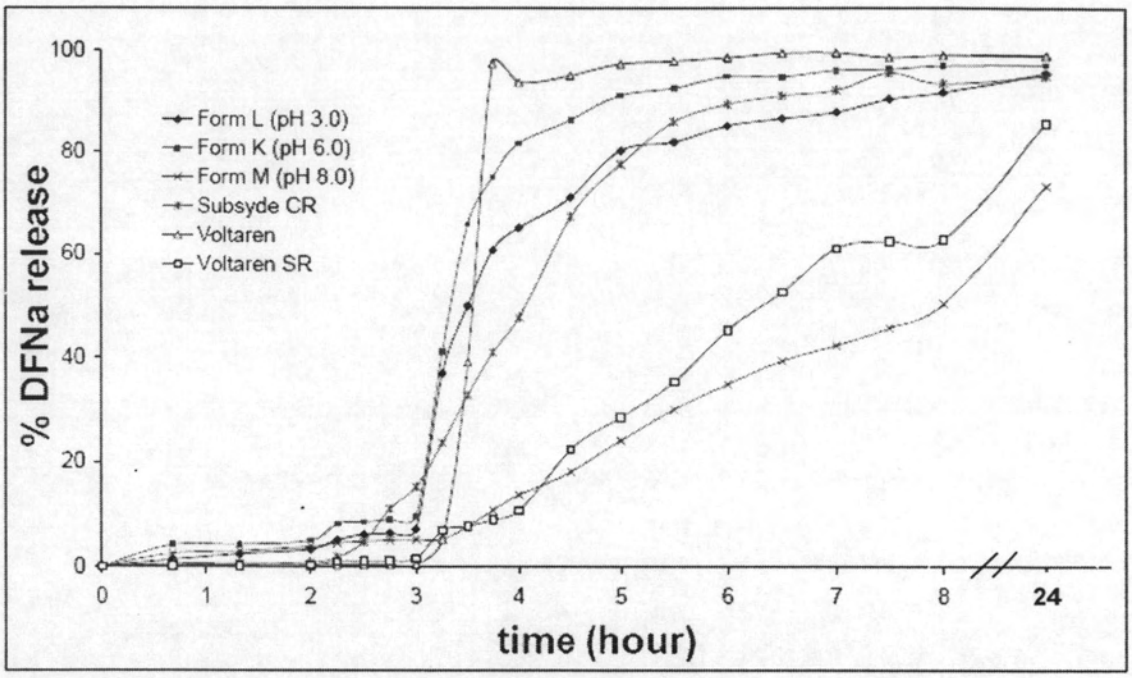


Figure 4.28 The dissolution profiles of sodium diclofenac from the beads with various pH values of coagulant solution in the pH-alternating system.

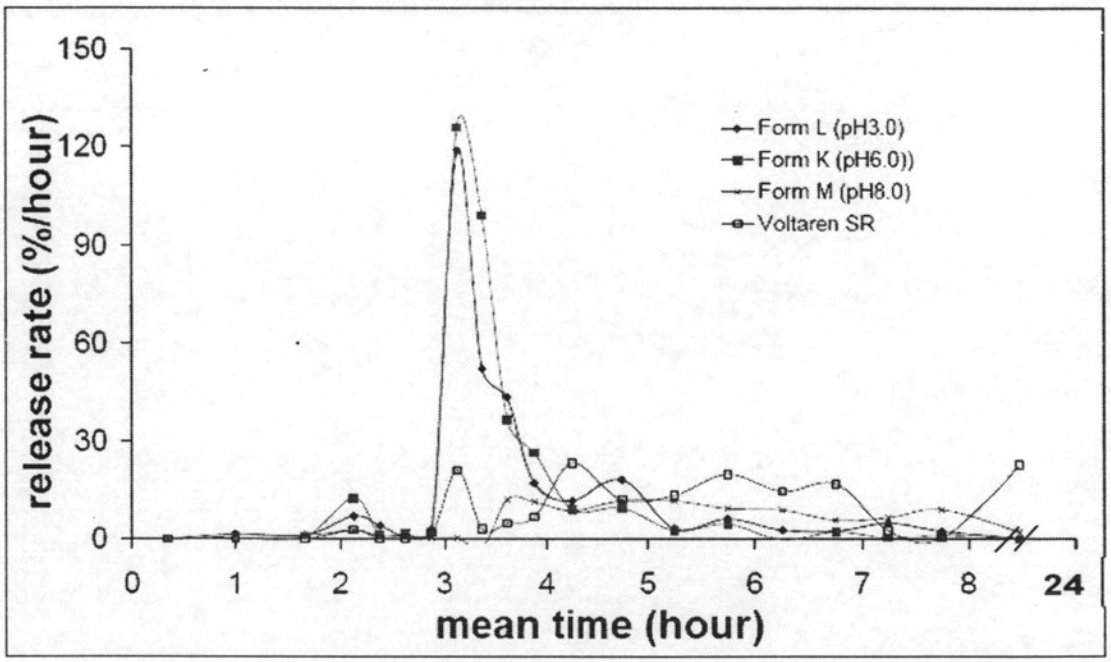


Figure 4.29 The release rate profiles of sodium diclofenac from the beads with various pH values of coagulant solution in the pH-alternating system.

Effect of the cross-linking time on the dissolution profiles

The cross-linking time is important for beads forming, %EE and % release of drug. Three formulations, Formulation K, N and O, had been prepared at the various cross-linking time, 20, 30 and 60 minutes, respectively. The dissolution profiles and release rate of those three formulations are presented in Figure 4.30 and 4.31. Also, the release rate profiles were shown in Figure 4.32. The percentage of DS release was presented in Table C1 (Appendix C). The acidic medium, pH 1.2, was used as dissolution medium at the initial period of the dissolution test. After 2 hours, the dissolution medium was changed to phosphate buffer pH 6.6. All three formulations were presented the little increase of drug release percentage in the slowly manner. For the next dissolution medium, the characteristics of dissolution profiles for Formulation K, N and O were not significantly different. Moreover, all Formulations also had the highest drug release and comparative encapsulation efficiency. However, Formulation N was selected for subsequent study because it took shorter time to process.

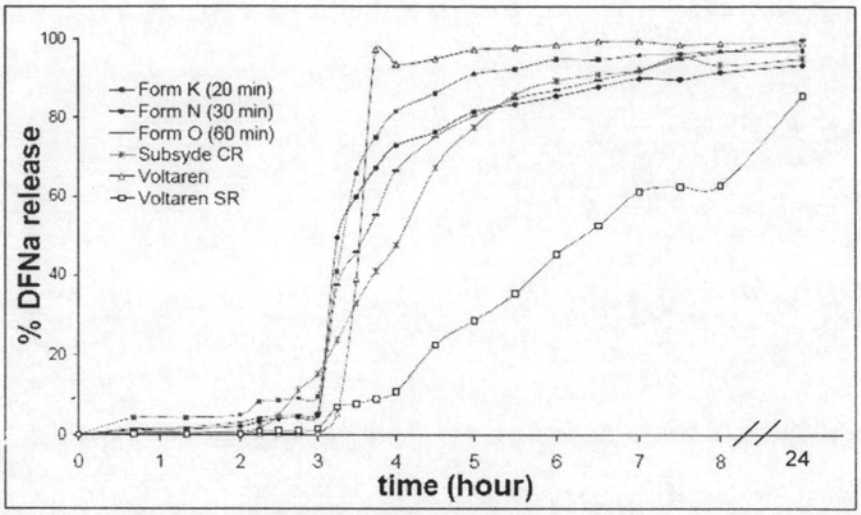


Figure 4.30 The dissolution profiles of sodium diclofenac from the beads with various cross-linking time in the pH-alternating system.

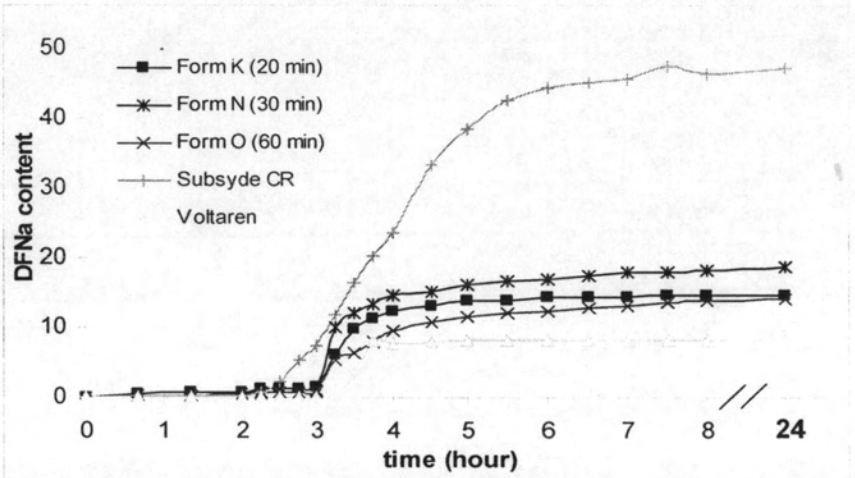


Figure 4.31 The dissolution drug content of sodium diclofenac from the beads with various cross-linking time in the pH-alternating system.

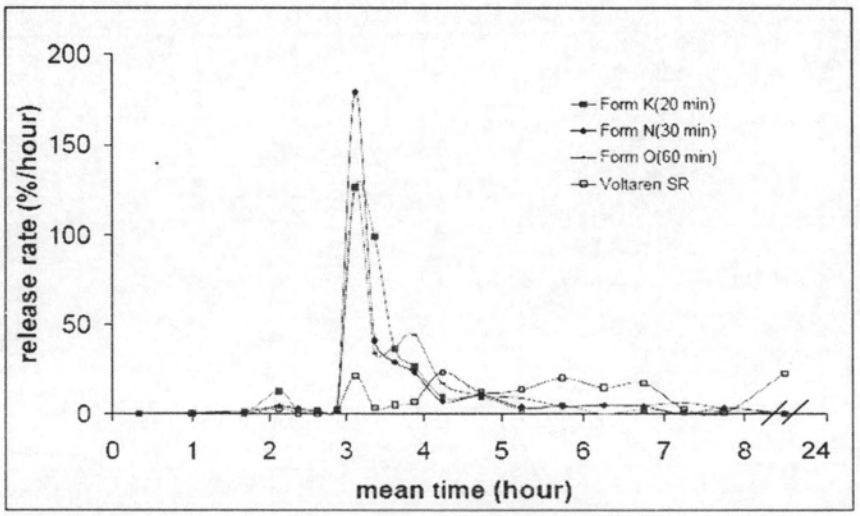


Figure 4.32 The release rate profiles of sodium diclofenac from the beads with various cross-linking time in the pH-alternating system.

Effect of the chitosan beads size on the dissolution profiles

Formulation N, prepared at 10% TPP at pH 6.0 for 30 minutes, was the most suitable formulation in chitosan bead preparation, gave high efficiency in %EE and % drug release. After that, the effect of the shape and size of beads were studied for evaluating the optimum condition in chitosan-TPP beads.

Formulation N, P and Q were represented the chitosan beads formulations, prepared from different sizes of the syringe needles; number 18, 22 and 24, respectively. (see Table 3.2, Chapter 3). This part, these three formulations were studied on the dissolution test and the dissolution profiles and release rate of those three formulations are presented in Figure 4.33and 4.34.

In the pH-alternating system, during the initial 3 hours of dissolution time, all formulations presented the low percentages of drug release, less than 3%. Swelling ration gave the direct effect onto the beads size. Formulation Q which had the smallest bead size displayed the lowest swelling ratio. While the bigger size of the beads in Formulation N and P reflected in higher swelling ratio also. But this result is insignificant when considering in release percentage just only first 2 hours.

After that, the rapid release of DS from each formulation was happened at 3rd hour for 30 minutes. Although, Formulation N and P presented the burst release but they gave the continuous released percentage until 24 hours. Because of the same condition of chitosan bead preparation, the drug releasing profiles of Formulation N and P were the same pattern. In contrast, Formulation Q was formed as same condition as both formulations, but it gave the poor dissolution profile. Because of the chitosan hydrogel drops, that prepared through syringe needle No. 24, was unreliable in sizes due to the viscosity. Thus, it gave the unexpected results in both % EE and %drug release.

From the dissolution percentages in Table C1 (Appendix C), 80% of entrapped DS will be released at 5th hour for Formulation N. Whereas Formulation P sustained release to 6th hour. Moreover, Formulation Q had stop release since 4th hour at 44.94%. In addition, both Formulation N and P could release more drug until 24th hour in the same pattern, but Formulation P gave slower DS release profile. Therefore, the syringe needle number 22 could be the used for improving to reduce beads and slow dissolution profile of chitosan beads.

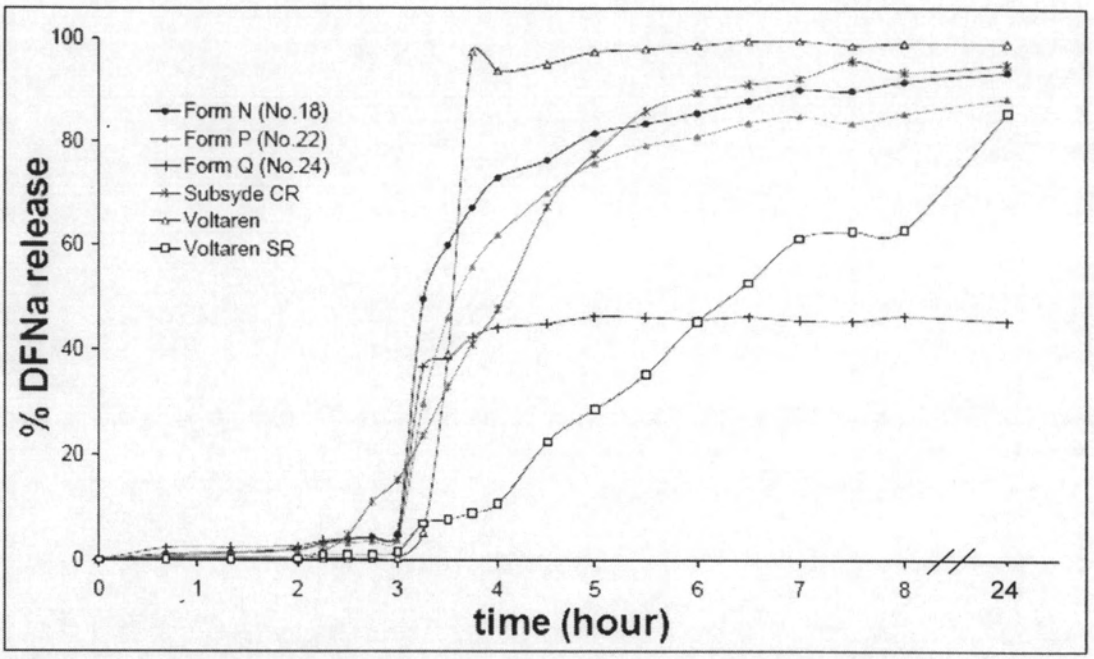


Figure 4.33 The dissolution profiles of sodium diclofenac from the beads with various syringe needles in the pH-alternating system.

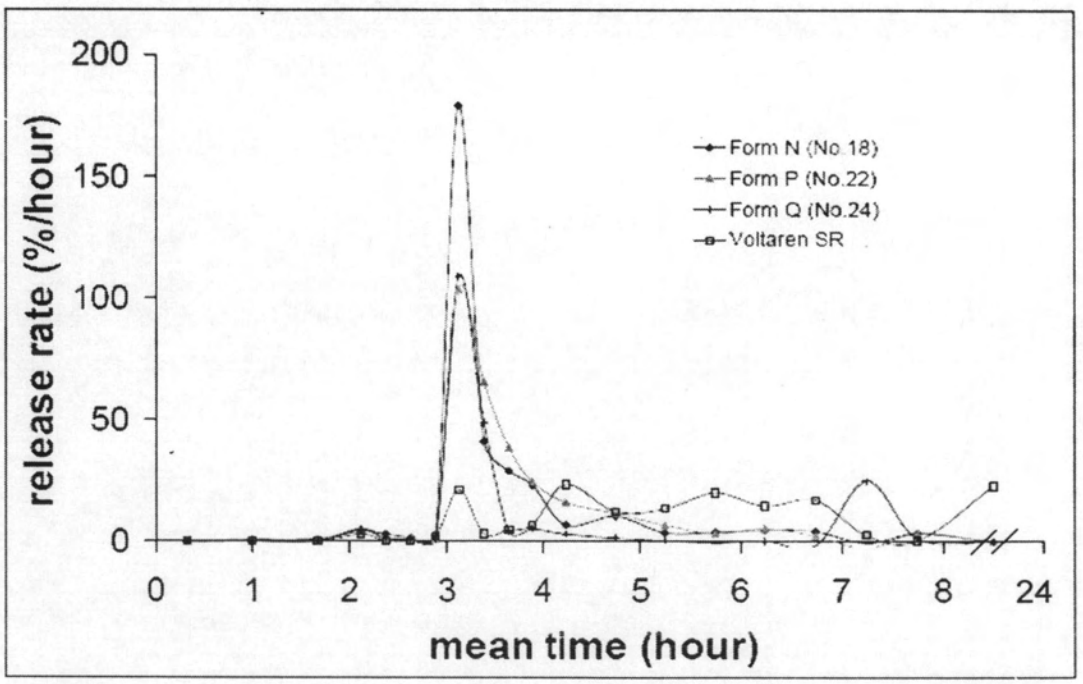


Figure 4.34 The release rate profiles of sodium diclofenac from the beads with various syringe needles in the pH-alternating system.

In previous studies, the beads formed by chitosan alone gave an unsatisfied sustained release profile. The formulation can be improved by forming a polyelectrolyte complex with PEG. Consequently, it is necessary to investigate (i) the ratio between chitosan and PEG, (ii) the ratio between polymer and drug and (iii) a crosslinking agent.

Effect of the polymer ratio (chitosan/PEG) on the dissolution profiles

The dissolution profiles of chitosan beads reinforced by PEG were studied in the pH alternating system. The dissolution profiles and the release rate of the various ratios of chitosan/PEG beads were illustrated at Figure 4.35-4.36. The dissolution profiles and the release rate of Formulations were presented in Table C1 (Appendix C). The various ratios of chitosan/PEG beads were studied, namely Formulation PEG1, PEG2, PEG3 and PEG4 which consisted of chitosan/PEG ratio 1:0.25, 1:0.5 1:1 and 1:2, respectively (see Table 3.2, Chapter 3). From the dissolution profiles show that PEG can help slowing the release rate. With more PEG, the release rate is slower. However, when the CS/PEG ratio is 1:2, the beads cannot firmly entrap the drug, resulting in the low drug release. This result consistent with the swelling property. The swelling ratio of the 1:2 CS/PEG beads is 1.51 which is higher than 1.25 of the CS beads, at first two hours. Consequently, the drug can be diffused out from the beads more easily.

Formulation PEG2 (1:0.5 CS/PEG) can sustain release the diclofenac within 24 hours with high encapsulation efficiency (92.10%) and can release all the encapsulated drug (92.66%).

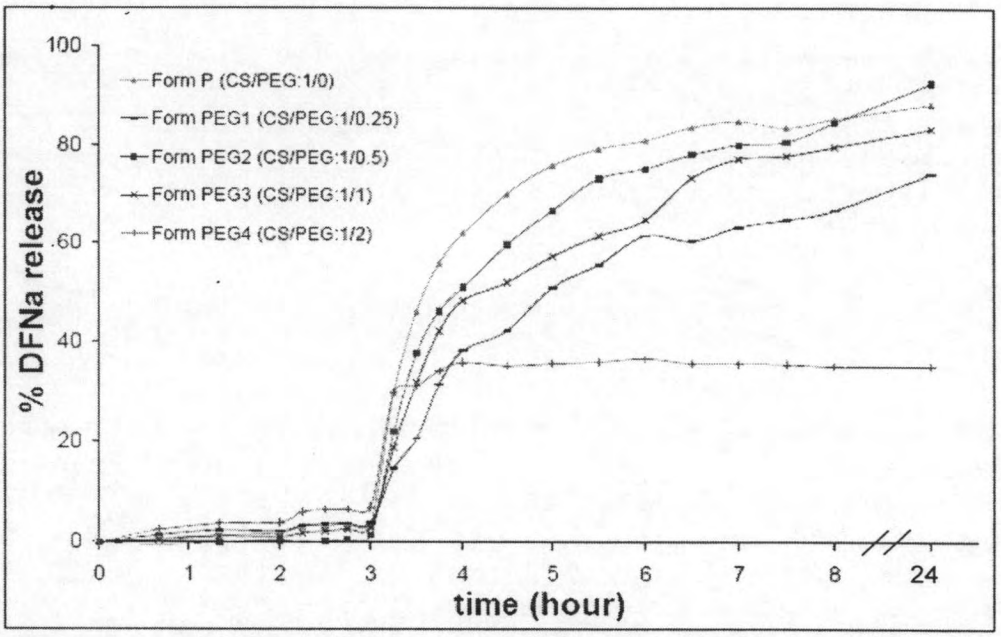


Figure 4.35 The dissolution profiles of sodium diclofenac from the beads with various chitosan/PEG ratios in the pH-alternating system.

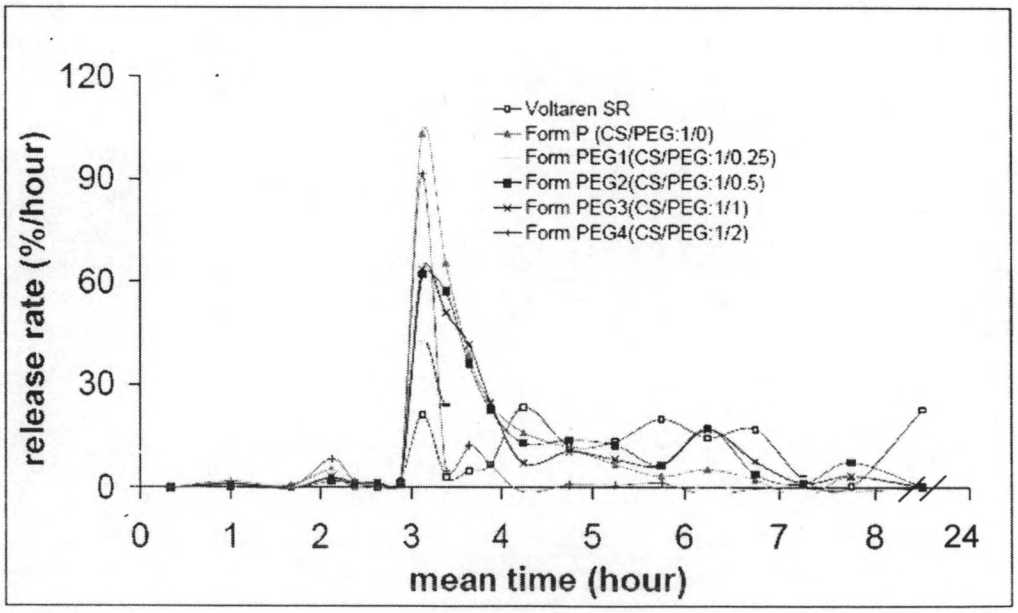


Figure 4.36 The release rate profiles of sodium diclofenac from the beads with various chitosan/PEG ratios in the pH-alternating system.

Effect of the polymer to drug ratio on the dissolution profiles

Once the suitable ratio of chitosan/PEG is obtained, the next parameter to be considered is the loaded amount of diclofenac. In this study, the CS/PEG polymer ratio was fixed at 1:0.5, and then the ratio between the polymer and drug was varied 1/0.25, 1/0.5, 1/1 and 1/1.5, namely Formulation PEG5, PEG2, PEG6 and PEG7, respectively (seeTable3.2, Chapter 3).

The dissolution profiles and the release rates of the various ratios of polymer /DS were illustrated in Figure 4.37-4.38, see Table C1 (Appendix C).

In the alternating system of dissolution test, the first three hours period of dissolution medium, after first 2 hours, drug release percentage of PEG5 was highest at 3.10% whereas, Formulation PEG6 and PEG7 had a very low drug release percentage at 0.83% and 1.24%. This happened because the higher percentage of DS effected to the lower swelling ratio of the bead. Therefore, lower swelling beads produced less drug release percentage.

After that, in the phosphate buffer saline pH 6.6 and 7.4, Formulation PEG5 presented the rapid release about the first hour and then it kept slightly increase up to 13% for 24^{th} hours. Whereas, the drug release for the other formulations slightly increase in first period of the dissolution test.

The release profiles of Formulation PEG2, PEG6 and PEG7 had the similar dissolution patterns. In addition, the initial burst effect was reduced. Formulation PEG2 gave the highest drug release of 92.66% within 24 hour, while the Formulation PEG6 and PEG7 showed only 69.97% and 68.10% of the drug release, respectively. When the amount of drug increase, the less drug content could be entrapped.

Formulation PEG2 can sustain release the diclofenac within 24 hours with high encapsulation efficiency (92.10%) and can release all the encapsulated drug (92.66%).

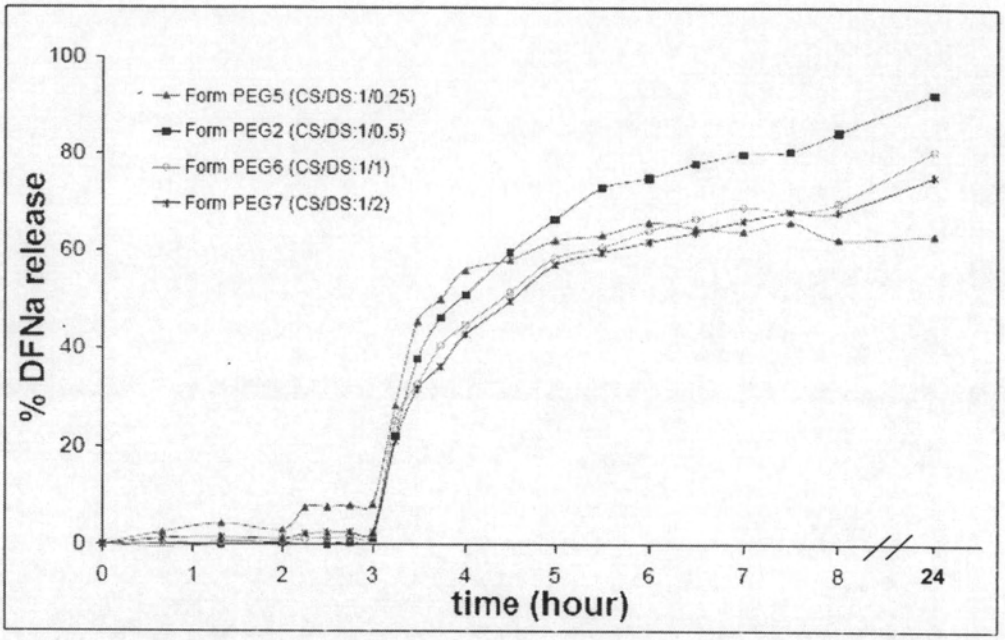


Figure 4.37 The dissolution profiles of sodium diclofenac from the beads with various polymer to DS ratios in the pH-alternating system.

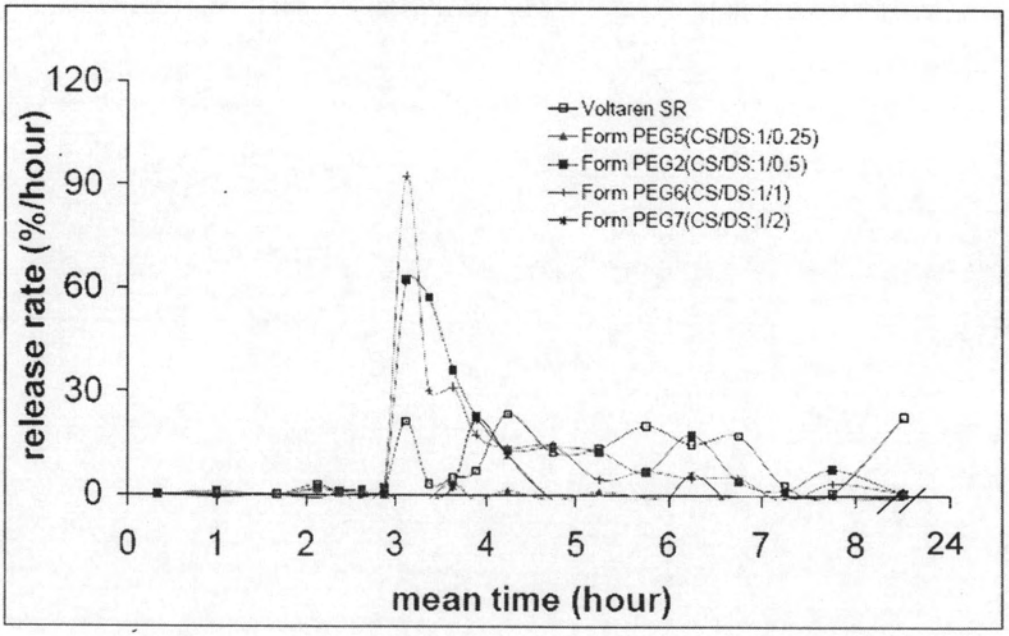


Figure 4.38 The release rate profiles of sodium diclofenac from the beads with various polymer to DS ratios in the pH-alternating system.

Effect of the cross-linking agent on the dissolution profiles

In this study, the effect of covalent crosslinking agent on the dissolution profiles were investigated. Glutaraldehyde (GD) is commonly used as a covalent cross-linking agent for enhancing the dissolution property of chitosan beads and was selected to a covalent crosslinking agent.

The Formulation GD2.5, GD5 and GD 7.5 were represented to chitosan/PEG cross-linked with 2.5% GD, 5% GD and 7.5% GD (see Table 3.2, Chapter 3). The dissolution profiles and the release rates of chitosan/PEG cross-linked with GD beads were presented at Figure 4.39 and 4.40, Table C1 (Appendix C).

The suitable formulation of the controlled drug release was studied from the dissolution test at the pH-alternating system. At first 2 hours, all three formulations of the chitosan/PEG GD beads presented the dissolution percentage at 5.57, 6.61 and 4.67%. After that, the dissolution medium was change to phosphate buffer saline pH 6.6 for 1 hour and pH 7.4 until 24 hours. The dissolution patterns of the three formulations were similar, that drug release was increased in 3^{rd} -4^{th} period and continuously increased of drug release were showed until 24^{th} hour.

At the 6^{th} hour, the dissolution percentage of the Formulation GD2.5, GD5 and GD 7.5 were 76.75, 69.57 and 69.46%, respectively. The release pattern of GD5 and GD7.5 is not so different. From Figure 4.39, it was seen that Formulation GD5 could release most of the entrapped drug at 99.61%, whereas GD7.5 gave the lower percentage of total release, 97.14% over 24 hours.

Compare GD5 (5% GD) to PEG2 (TPP alone), the release rate of diclofenac can be well controlled by GD.

Therefore, Formulation GD5 is the most suitable formulation to use as a controlled release formulation for DS. In addition, the drug release pattern of this formulation was close to that of the sustained commercial drug of Voltaren SR.

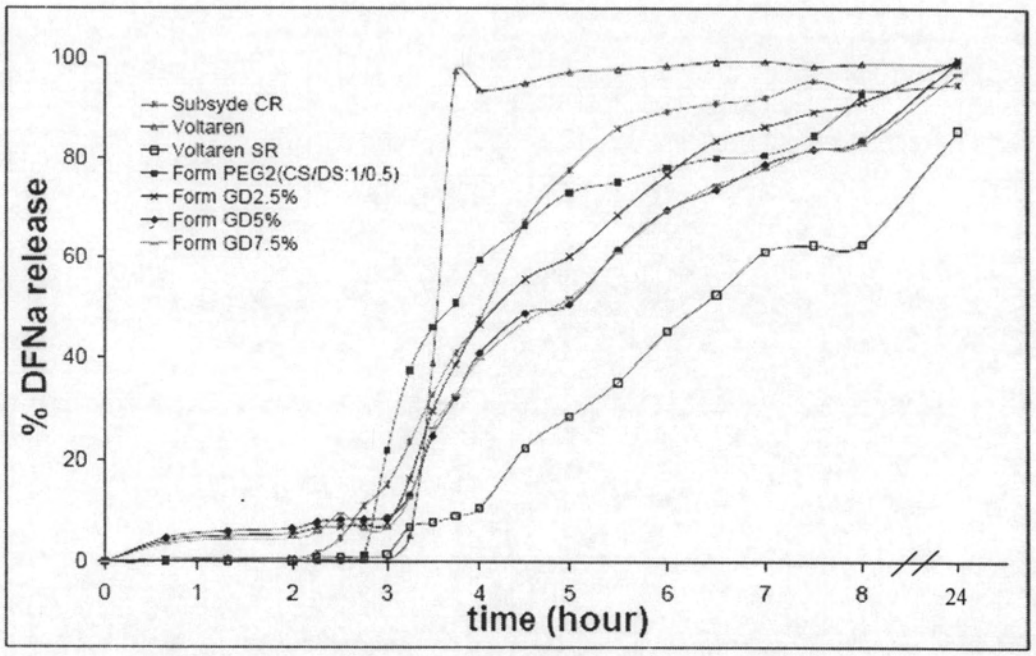


Figure 4.39 The dissolution profiles of sodium diclofenac from the beads with various concentration of crosslinking agent in the pH-alternating system.

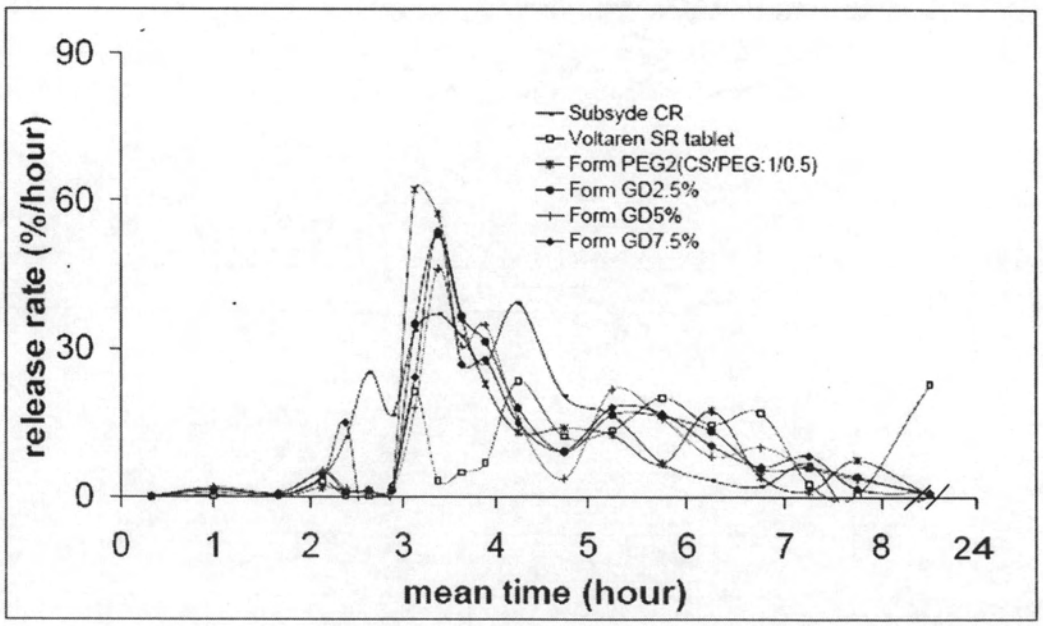


Figure 4.40 The release rate profiles of sodium diclofenac from the beads with various concentration of crosslinking agent in the pH-alternating system.