SHORT COMMUNICATION

Development of indomethacin sustained release microcapsules using chitosan-carboxymethyl-cellulose complex coacervation

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Abstract

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Development of indomethacin sustained release microcapsules using chitosan-carboxymethylcellulose complex coacervation

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Indomethacin sustained release microcapsules were prepared by complex coacervation of chitosan (CS) and carboxymethylcellulose (CMC) and then were hardened with glutaraldehyde (GA). The effects of concentration and pH of CS solution, amount of GA and hardening time on the physicochemical properties and drug release of these microcapsules were investigated. The SEM photomicrographs revealed that surface morphology of microcapsules depended on the pH of CS solution. Decreasing the pH increased the smoothness of the surface due to the relaxation of CS chain in acidic medium. The geometric mean diameters of the microcapsules were between 126-212 microns. Those prepared from CS solution of pH 4 and hardening time of 3 hours seemed to have the narrowest size distribution. The percent drug entrapment was comparable in the range of 40%-50% while the percent drug recovery varied between 60%-87%. The latter increased when decreasing the pH and increasing the concentration of CS solution but decreased when increasing the hardening time. Dissolution study showed that microcapsules prepared from CS solution of high pH initially

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released the drug faster than those from CS solution of lower pH. After 3 hours their release rate was similar. Increasing the amount of GA and hardening time decreased the drug release due to denser membrane. In contrast, the concentration of CS solution had no effect on drug release. The mechanism of drug release was prominently diffusion controlled through wall membrane and pore. The kinetics of drug release followed Higuchi's model.

Key words : indomethacin, microcapsule, chitosan, carboxymethylcellulose

บทคัดย่อ

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การพัฒนาไมโครแคปซูลออกฤทธิ์นานอินโดเมธาซิน โดยใช้โคอะเซอร์เวชันเชิงซ้อนของไคโตซาน-คาร์บอกซีเมธิลเซลลูโลส

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ไมโครแคปซูลชนิดออกฤทธิ์นานอินโดเมธาซินเตรียมได้ด้วยวิธีโคอะเซอร์เวชันเชิงซ้อนของไคโตซานและคาร์บอกซีเมธิลเซลลูโลส แล้วทำให้แข็งแรงด้วยกลูตาราลดีไฮด์ และศึกษาผลของความเข้มข้นและพีเอชของสารละลายไคโตซาน ปริมาณของกลูตาราลดีไฮด์ และเวลาในการทำให้แข็งแรงต่อคุณสมบัติทางเคมีฟิสิกส์และการปลดปล่อยยาจากไมโครแคปซูล รูป SEM แสดงว่าลักษณะผิวของไมโครแคปซูลขึ้นกับพีเอชของสารละลายไคโตซาน การลดพีเอชจะเพิ่มความเรียบของผิวเนื่องจากการคลายตัวของสายโซ่ไคโตซานในตัวกลางที่เป็นกรด ค่าเฉลี่ยของเส้นผ่าศูนย์กลางเชิงเรขาคณิตจะอยู่ระหว่าง 126-212 ไมครอน ไมโครแคปซูลที่เตรียมจากสารละลายพีเอช 4 และเวลาในการทำให้แข็งแรง 3 ชั่วโมงจะให้การกระจายอนุภาคน้อยที่สุด เปอร์เซ็นต์ยาที่กักเก็บในไมโครแคปซูลมีค่าใกล้เคียงกันระหว่าง 40%-50% ในขณะที่เปอร์เซ็นต์ยาที่เก็บคืนจากการเตรียมจะแปรปรวนระหว่าง 60%-87% โดยจะเพิ่มเมื่อลดพีเอชและเพิ่มความเข้มข้นของสารละลายไคโตซาน แต่จะลดเมื่อเพิ่มเวลาในการทำให้แข็งแรง การศึกษาการละลายพบว่า ไมโครแคปซูลที่เตรียมจากสารละลายพีเอชสูงจะปลดปล่อยยาในระยะเริ่มแรกเร็วกว่าจากที่เตรียมจากสารละลายพีเอชต่ำ อย่างไรก็ตามหลัง 3 ชั่วโมง อัตราการปลดปล่อยยาจะใกล้เคียงกัน การเพิ่มปริมาณกลูตาราลดีไฮด์และเวลาในการทำให้แข็งแรงจะลดการปลดปล่อยยา เนื่องจากเมมเบรนที่แน่นกว่า ในทางตรงกันข้ามความเข้มข้นของสารละลายไม่มีผลต่อการปลดปล่อยยา กลไกการปลดปล่อยยาจะถูกควบคุมโดยการแพร่ผ่านผนังและรูเปิด จลนศาสตร์ของการปลดปล่อยยาจะเป็นไปตามรูปแบบของฮิกูชิ

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Microcapsules containing a drug core could be prepared by aqueous complex coacervation of chitosan (CS) and various counter polyanions (Kim and Rha, 1989; Shioya and Rha, 1989; Takahashi *et al*., 1990; Meshali and Gabr, 1993; Ohya *et al*., 1993; Polk *et al*., 1994; Remunan-Lopez and Bodmeier, 1996). Their permeability characteristics were theoretically controlled by the physical and processing conditions that contribute to the polymer chain conformation in solution. Therefore, it was possible to control the micro-structure and mechanical properties of CS membrane by manipulating the solution conditions. The decreasing of the molecular weight of CS was reported to decrease the permeability of cell culture from CS-carboxymethylcellulose (CMC) microcapsule (Shioya and Rha, 1989). The pH, ionic strength and polymer concentration of CS solution were shown to affect the permeability of CS-sodium alginate microcapsule (Kim and Rha, 1989; Shioya

and Rha, 1989; Ohya *et al*., 1993; Remunan-Lopez and Bodmeier, 1996). Crosslinking agents were reported to reduce the degree of swelling and the rate of drug release from crosslinked chitosan microcapsule (Hou *et al*., 1985; Thanoo *et al*., 1992; Polk *et al*., 1994; Remunan-Lopez and Bodmeier, 1996). The concentration of anionic polymer and processing temperature also had an effect on the formability of CS-CMC microcapsules (Ritthidej and Tiyaboonchai, 1997). Encapsulation of an acidic drug, indomethacin, was noted but not pindolol which was a basic drug.

Indomethacin, a nonsteroidal anti-inflammatory agent, has a short biological half-life of 2.6-11.2 hours (Flower *et al*., 1990). The usual oral dosage for adults is 25 or 50 mg, 2 to 3 times a day. Controlled release preparations of this drug are to increase patient compliance and to reduce adverse effects, fluctuation in plasma concentration and dosing frequency. Therefore, the objective of this study was to investigate the preparation of complex coacervation of CS and CMC to control the release of indomethacin from microcapsule. The effects of concentration and pH of CS solution, the amount of glutaraldehyde (GA), a crosslinking agent and the hardening time on the physicochemical properties and drug release pattern of the pharmaceutical microcapsule were studied.

Experimental

Materials: The following materials obtained from commercial sources were used as received: indomethacin (Batch# 850602, China), CS (MW 30,000-50,000, distributed by G.T. Chemical Co. Ltd., Thailand), CMC (lot # 7532C6, distributed by Bhaesachpanit Co. Ltd., Thailand), GA (lot # 1-83M2, Union Carbide, USA), glacial acetic acid (E. Merck, Germany).

Microencapsulation method: Indomethacin microcapsules were prepared as previously reported (Ritthidej and Tiyaboonchai, 1997) by spraying 1% w/v CMC solution containing 1% w/v of indomethacin through a nozzle by peristaltic pump (UniGlatt Laboratory unit, Germany) at 1.2 rpm with 1.5 psi air pressure (air pump serial #357552, USA), into a bath of CS solution maintained at 15±2ºC. Different CS concentrations of 0.25 to 1.0% w/v in 1.0% w/v acetic acid solution and having pH of 3, 4 and 5 were studied. GA of 0.5 to 2.0 g/g of polymer was added. The mixture was continuously stirred (magnetic stirrer, model M21/1, Framo, Italy) at 650 rpm for 1 to 5 hrs. The hardened microcapsules were filtered, washed with water and then with isopropanol before drying under nitrogen gas. The dried microcapsules were passed through a #40-mesh sieve and collected. The yield microcapsules were then filled into capsules #0 without other additive. Each capsule contained 50 mg of indomethacin.

Evaluation of indomethacin microcapsule:

1. Morphology. The morphology of microcapsules was determined by scanning electron microscopy (JSM-T220A, Jeol, Japan). Each sample was coated with gold prior to the microscopic examination using ion sputtering. Shape and surface morphology were observed. Selected preparations were observed for their surface morphology both before and after drug release study.

2. Size and size distribution. The size and size distribution were determined from SEM photomicrographs. The particle size was determined by measuring the Martin's diameter (Martin, 1993). Sample size of about 350 microcapsules was used for size distribution analysis. The cumulative percentage frequency undersize and normalized Z values were calculated from the number of particle size distribution. The geometric mean diameter at Z=0 or D_{50} of microcapsule was then computed from the least square analysis of the logarithm of particle diameter and Z value.

3. Percentages of drug entrapment and drug recovery. The percentage of drug entrapment was calculated from the content of indomethacin in microcapsule determined according to the monograph of Indomethacin Extended Release Capsule in the USP XXIV. The percentage of drug recovery was computed from the following equation.

$$\% \text{ Recovery} = M_t.D_m 100/M_a D_i \quad ....(1)$$

where D_m, M_t, D_i and M_a were the drug content in microcapsule, the weight of total microcapsule yield and the initial amount of drug used in the microencapsulation process and weight of the determined microcapsule, respectively.

4. Drug release study. The drug release from microcapsule was determined according to the USP XXIV by using a dissolution apparatus I (Model SR2, Hanson Research, USA). Nine hundred ml of phosphate buffer of pH 6.2 equilibrated at 37.0±0.5ºC was used as dissolution medium. The apparatus was operated at the rotating speed of 75 rpm. The amount of indomethacin release at any time interval was determined spectrophotometrically at 320 nm (Spectronic 2000, Bausch & Lomb, USA) and calculated from the calibration curve. The cumulative correction was also conducted.

Results and Discussion

Microencapsulation method

Indomethacin microcapsules were satisfactorily produced. However, some preparations could not be collected in the recovery process. They either adhered together to form tacky agglomerate or were trapped in the gelled medium. Collapsing was evident in microcapsules prepared from CS solution of pH 3 during the hardening period. Those prepared from CS of pH 5 subsequently burst when washing with isopropanol. Microcapsules prepared from CS solution of higher pH tended to swell and took longer drying time. The dried microcapsules were pale yellowish and looked like granules. Those prepared from CS solution of lower pH showed better flowability than those of higher pH.

Morphology. Figure 1 shows the shape and surface topography of microcapsules prepared from various formulations and processing conditions under SEM. It could be clearly seen in Figures 1a-1c that the pH of CS solution played a prominent role on the surface morphology of the obtained microcapsules. Smooth and wavy surface was found on microcapsules prepared from solution of pH 3. Those from pH 4 showed rough and wrinkled membrane while those from pH 5 were fibrous membrane. The result was owing to the conformation of CS in preparation medium. At lower pH, CS was more soluble (Hou *et al*., 1985). The configuration of polymer chains was more relaxed and unfolded. Therefore ionic interaction with CMC molecule was easier and more complete. Consequently, smoother surface was obtained.

The amount of GA also had an effect on the surface morphology of microcapsules. The surface of microcapsules became denser when increasing the amount of cross-linking agent as shown in Figures 1a, 1d-1e. GA could react with hydroxyl group in CMC chain to form acetal and reacted with amino group in CS to form schiff's base (Hou *et al*., 1985). The cross-linking resulted in more rigid membrane, thus denser and smoother surface of microcapsule. However, cracking could be noticed when using GA of 1.5 g. This was seemingly due to the lower flexibility of membrane at such high amount of cross-linking agent. Hardening time also had some effect on surface morphology. Although similar wavy surface was shown at any experimental hardening time for microcapsules prepared from CS solution of pH 3 and GA of 1.0 g as shown in Figures 1g-1i, cracking could be noticed at long hardening time. In contrast, the concentration of CS solutions seemed to have no effect on the shape and surface morphology of the obtained microcapsules as shown in Figures 1b and 1f. The resulting microcapsules exhibited similarly rough and wrinkled surface.

Size and size distribution. The geometric mean diameters at Z=0, D_{50}, of the prepared microcapsules are listed in Table 1. They were between 126.11 to 212.23 μm. Microcapsule from CS solution of pH 5, GA of 1.0 g and hardening time of 1 hour was the smallest. This was close to the reported CS-CMC microcapsules prepared from pH 4, GA of 0.25 g and hardening time of 3 hours (Ritthidej and Tiyaboonchai, 1997). The size of microcapsules seemingly depended on the investigated parameters. Although GA content did not have an effect on the size of microcapsule when

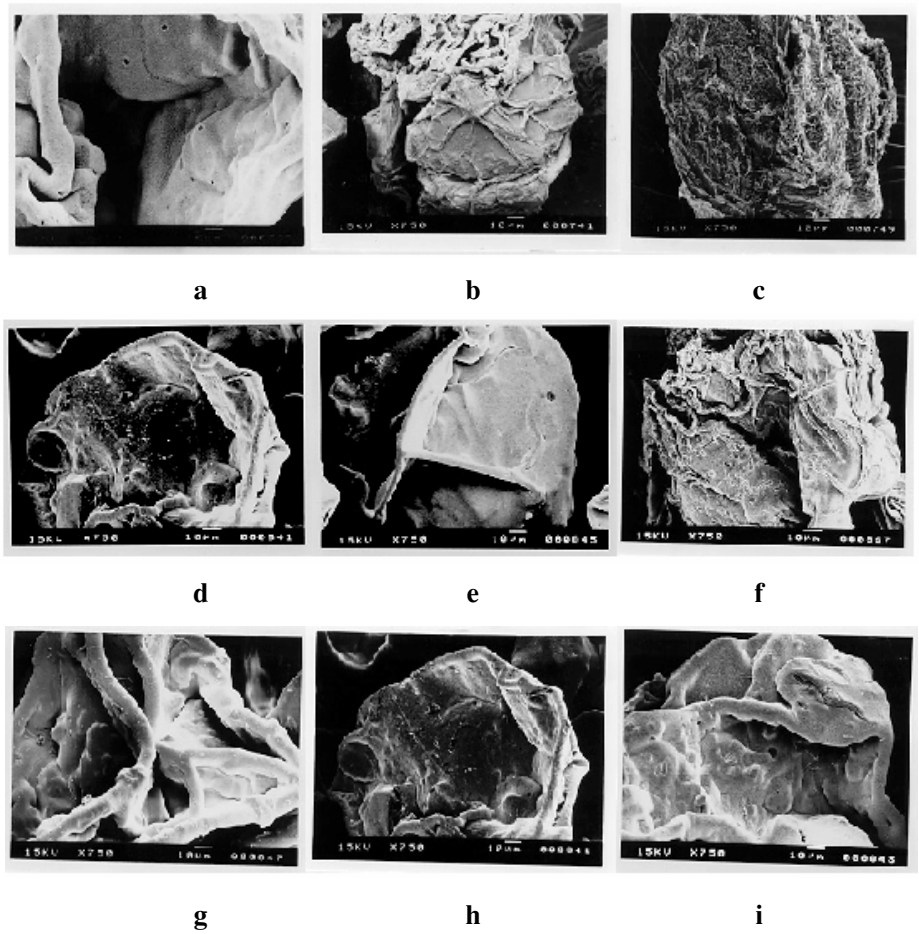


Figure 1. SEM of indomethacin microcapsules prepared from 0.5% CS solution of pH 3 (a), pH 4 (b), pH 5 (c) with glutaraldehyde of 0.25 g, hardening time of 3 hours; from CS solution of pH 3, hardening time of 3 hours and glutaraldehyde of 1.0 g (d), 1.5 g (e); from CS solution of pH 4 glutaraldehyde of 0.25 g, hardening time of 3 hour and 1.0% CS solution (f); from CS solution of pH 3, glutaraldehyde of 1 g and hardening time of 1 hour (g), 2 hours (h), and 5 hours (i).

using low pH of CS solution, increasing the content of GA significantly increased the size at higher pH of CS solution. As aforementioned, CS in medium of low pH completely dissolved and interacted with the cationic polymer. At higher pH of CS solution, CS was less soluble or had less relaxed configuration, hence less NH_3^+ to react with CMC. Availability of NH_3^+ to react with GA was also less. Polymerization of excess GA in the medium of high pH could occur (Richter, 1952) and eventually increased the viscosity of the medium. It was evident that microcapsules could not be formed at high amount of GA and CS solution of pH 5 due to the gelation of the medium. Although the size of microcapsules was prominently dictated by the droplet size of drug-CMC dispersion (Polk *et al*., 1994; Ritthidej and Tiyaboonchai, 1997), incomplete complex coacervation would result in either aggregates or viscous medium, which would enlarge the size. Nixon and Hassan (1989) reported that hardening with formaldehyde slightly enlarged the microcapsule

Table 1. Formulation and processing variables affecting the size, drug entrapment and drug recovery in indomethacin microcapsules.

Conc. of Cs solution (%)	pH of CS solution	Hardening time (hour)	GA/polymer (g)	D_{50}* (μm)	% Drug entrapment** ±S.D.	% Drug recovery** ±S.D.
0.5	3	5	1.0	187.15	40.25±1.51	74.18±2.77
0.5	3	5	1.5	151,11	40.06±1.59	75.36±2.99
0.5	3	5	2.0	170.46	43.31±3.76	86.67±3.76
0.5	3	3	0.5	166.46	43.38±0.77	79.38±1.16
0.5	3	3	1.0	163.22	41.44±1.30	74.51±2.34
0.5	3	3	1.5	170.69	40.00±3.75	69.95±6.50
0.5	3	3	2.0	155.78	42.42±1.82	77.78±3.34
0.5	3	1	1.0	194.75	45.54±0.52	81.63±0.94
0.5	4	1	0.5	174.35	46.56±5.46	69.30±8.15
0.5	4	1	1.0	146.93	42.90±4.51	64.73±6.81
0.5	4	1	1.5	204.24	42.38±2.11	66.23±3.30
0.5	4	3	0.5	143.80	41.12±2.05	71.08±3.54
0.5	4	3	1.0	184.95	43.08±0.58	71.18±0.97
0.5	4	3	1.5	212.23	45.38±0.51	75.25±0.84
0.5	5	1	0.5	199.77	48.35±0.47	63.94±0.62
0.5	5	1	1.0	126.17	44.54±0.73	66.22±1.09
0.5	5	3	0.5	174.76	41.29±1.11	50.98±3.53
0.25	4	3	0.25	185.5	39.13±1.43	60.72±2.93
0.75	4	3	0.25	160.4	43.98±0.62	77.04±1.10
1.0	4	3	0.25	150.3	45.92±1.55	80.29±2.69

* The geometric mean diameter at Z=0.
** Average from 3 determinations.

and caused a wider size distribution because of strengthened wall which did not reduce in thickness during the recovery process. The concentration of CS solution also had a slight effect on the size of microcapsule.

The size distribution of the prepared microcapsules was mostly between 63 to 311 μm with the high percentage weight between 126 to 212 μm. However, some preparations showed wider size distribution of 32 to 404 μm. Figure 2 compares the size distribution of microcapsules prepared from CS solution of different pH, amount of GA, hardening time and concentration of CS. The size distribution of the obtained microcapsules could be ranked: from CS solution of pH 4 < pH 3 < pH 5 as shown in Figure 2a, from hardening time of 3 hours < 1 hour < 5 hours in figure 2b, CS concentration of 0.5% < 0.75%< 1.0% < 0.25% in Figure 2d. In addition increasing the amount of GA increased the size distribution of micro-capsules as illustrated in Figure 2b. It was also noticed that multi-modal size distributions were obtained from solution of pH 5, GA $\geq$ 1 g and CS $\geq$ 0.75%. The results were likely due to the increasing viscosity of the medium that may cause deviation from the normal size distribution. At low CS concentration of 0.25%, multi-modal size distribution was also noted. This was probably due to the less reaction between of CS and CMC that resulted in apparent tackiness and agglomeration of the microcapsules. Therefore, optimum formulation and conditions have to be conducted in order to have narrow and normal size distribution.

Drug entrapment and drug recovery. Drug entrapment in CS-CMC microcapsules was within a narrow range of 39.13% to 48.35% as listed in Table 1. The pH of CS solution and reaction time seemed to have a slight effect on the drug entrap-

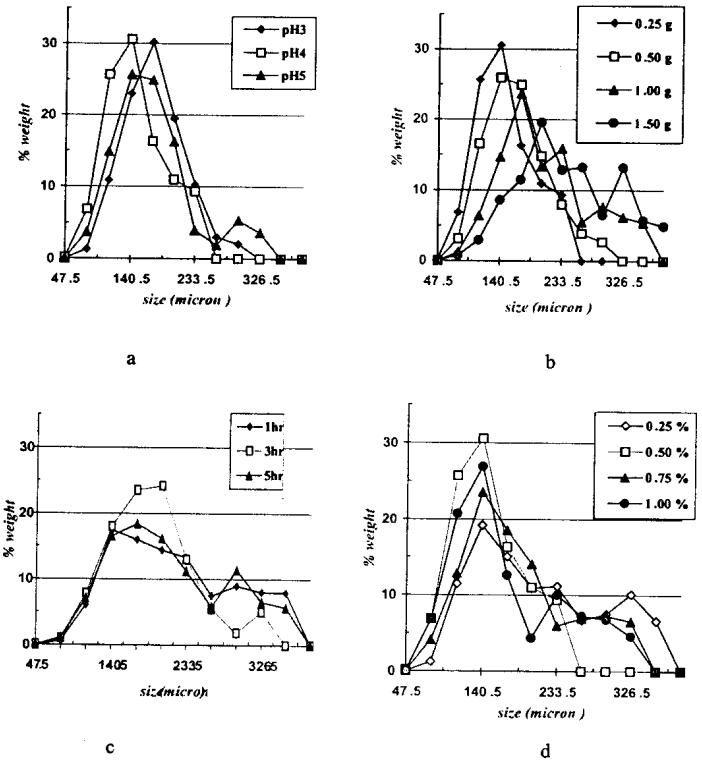


Figure 2. Size distribution of indomethacin microcapsule prepared from CS solutions of different pH with glutaraldehyde of 0.25 g and hardening time of 3 hours (a), different amounts of glutaraldehyde with CS solution of pH 4 and hardening time of 3 hours (b), different hardening times with CS solution of pH 3 and glutaraldehyde of 1.0 g (c), and different concentrations of CS solutions of pH 4, glutaraldehyde of 0.25 g and hardening time of 3 hours (d).

ment during the microencapsulation process. In contrast, the percentage of drug recovery shown in Table 1 was obviously varied between 50.98 to 86.67%. It could be seen that the drug recovery was influenced by the pH of CS solution. Microcapsules prepared from CS of lower pH were likely to achieve greater drug recovery than those from higher pH. This was obviously due to the solubility of drug. Indomethacin as an acidic drug was more soluble at high pH. However, viscous medium at high pH was noted when adding high amount of GA to CS solution. As a result, encapsulation was less efficient and may be highly varied as shown in preparations from CS solution of pH 3, GA of 1.5 g and hardening time of 3 hours and from CS solution of pH 4, GA of 1 g and hardening time of 1 hour. It was also noted that high drug recovery could be achieved by increasing the concentration of CS solution.

Drug release study. The effect of pH and concentration of CS solution, amount of GA, and hardening time on the release on drug from microcapsules is graphically presented as Higuchi's plots of the amount of drug released against square root of time as shown in Figure 3. It can be seen that the release of drug could be prolonged for 24 hours.

The effect of pH of CS solution on drug release is shown in Figure 3a. It is apparent that during the first hour, the drug release from microcapsules prepared with solution of pH 5 was the highest. This was likely due to the incomplete

complex coacervation shown as fibrous membrane of the microcapsule prepared from high pH of CS solution as aforementioned. Then the release gradually decreased. At this stage, the capsule wall was completely swollen, resulting in high tortuosity and greater effective diffusion path length in membrane that would retard the drug release from these preparations. Its release rate was eventually parallel to those prepared from solution of lower pH. It could be seen that after 3 hours, the pH of CS solution had no effect on the release rate and pattern in drug release study.

Figure 3b shows the effect of GA on the release of indomethacin from microcapsules. Increasing the content of GA decreased the release of drug to a certain level then the release was increased. This corresponded to the dense membrane when GA was added as previously shown. The amount of GA that achieved minimum drug release seemed to be 1.0 g with solution of pH 3. Addition of GA strengthened and decreased the porosity of the microcapsule wall during hardening process (Hou *et al*., 1985). Further increasing the GA content increased the drug release. This was due to excessive hardening treatment that caused the membrane to become brittle and crack (Nakatsuka and Andrady, 1992). This was in agreement with other investigators (Luzzi and Gerraughthy, 1967).

Hardening time also affected the release of indomethacin from CS-CMC microcapsules as shown in Figure 3c. When using CS solution of pH 3, the drug released from microcapsules prepared with hardening time of 1 hour was higher than that

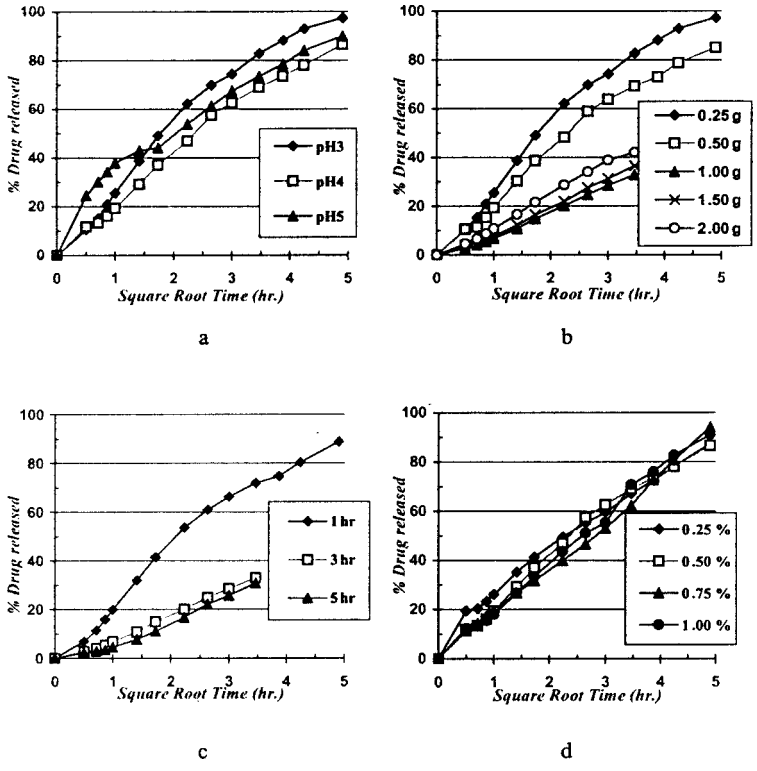


Figure 3. Higuchi's plot of indomethacin microencapsules prepared from CS solutions of different pH with glutaraldehyde of 0.25 g and hardening time of 3 hours (a), different amounts of glutaraldehye with CS solution of pH 3 and hardening time of 3 hours (b), different hardening times with CS solution of pH 3 and glutaraldehyde time of 1 g (c) and different concentrations of CS solution of pH 4, glutaraldehyde of 0.25 g and hardening time of 3 hours (d).

with longer hardening time.

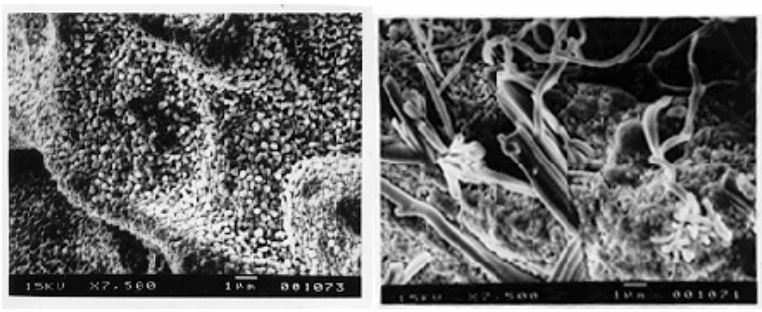
The concentration of CS solution appeared to slightly affect the release of drug as shown in Figure 3d. Slightly high drug release was found for the first 9 hours from microcapsules prepared from the lowest concentration of 0.25% w/v of CS solution. Other concentrations ranging from 0.5 to 1.0% exhibited similar release profile. No significant difference was found in Higuchi's plot of each preparation ($p \geq 0.05$). Kim and Rha (1989) demonstrated that the diffusion rate of bovine serum albumin decreased with the increase in CS concentration. However, they stated that the difference of the diffusional pattern was not large enough.

The analysis of SEM photomicrographs of microcapsule before and after dissolution study as shown in Figure 4 and the kinetic pattern showed that the release of indomethacin from microcapsule followed a mass transport phenomenon. The wall membrane of the microcapsule showed matrix properties, such as porosity and tortuosity that fitted the granular type matrix. It swelled after immersion into dissolution medium. This result was in agreement with other reports (Polk, 1994; Deasy, 1984). The release mechanism involved the permeation of dissolution medium through the wall membrane then the drug was dissolved and diffused through the membrane.

The calculated correlation coefficient of the % drug release versus square root of time was between 0.9376-0.9976 whereas those of the % drug release versus time and log % drug remained versus time were between 0.8050-0.9948 and 0.5132-0.8917, respectively. This implied that the release of drug from the prepared microcapsules accordingly followed Higuchi model.

Conclusion

The membrane of microcapsules was formed by electrostatic interaction between positive charged amine on the CS chain and the negative charged hydroxyl group on the CMC chain. GA reacted with hydroxyl group in CMC chain to form acetal and reacted with amino group in CS to form Schiff base. The crosslinking provided dense and rigid surface of microcapsule. Both hardening time and GA complementarily affected the strength of microcapsule wall. Moreover, GA or completion of cross-linking seemed to tighten the membrane thus causing smaller microcapsule. The pH of CS solution had prominent effect on the appearance, size and size distribution of microcapsules. The condition of CS solution obviously affected the drug recovery but not the drug entrapment. Higher pH of CS solution resulted in lower percentage of drug recovery. This was primarily due to the solubility of drug in the medium. In the drug release study, it could be concluded that the kinetic pattern of indomethacin microcapsules followed Higuchi's model. The pH of CS solution and GA content and hardening time affected the drug



a b

Figure 4. SEM of indomethacin microcapsules prepared from CS solution of pH 4, glutaraldehyde of 0.5 g and hardening time of 3 hours before (a) and after (b) dissolution study.

release kinetic of the yielded microcapsule but not the concentration of CS solution. The release of drug from microcapsule could be governed by optimizing the pH of CS solution, the hardening time and the GA content.

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