

Novel design of osmotic chitosan capsules characterized by asymmetric membrane structure for in situ formation of delivery orifice

Gen-Ming Wang^a, Chien-Ho Chen^b, Hsiu-O Ho^c, Sheng-Shi Wang^c, Ming-Thau Sheu^{c,*}

^a Department of Internal Medicine, Shin Kong Wu Ho-Su Memorial Hospital, 95 Wenchang Road, Taipei, Taiwan, ROC

^b School of Medical Technology, Taipei Medical University, 250 Wu-Hsing Street, Taipei, Taiwan, ROC

^c Graduate Institute of Pharmaceutical Sciences, Taipei Medical University, 250 Wu-Hsing Street, Taipei, Taiwan, ROC

Received 2 September 2005; received in revised form 24 March 2006; accepted 28 March 2006

Available online 7 April 2006

Abstract

In this study, chitosan capsules with asymmetric membrane to induce osmotic effects and in situ formation of the delivery orifice were optimally prepared and characterized. Chitosan capsules were formed on stainless steel mold pins by dipping the pins into a chitosan solution followed by forming asymmetric structure by dipping into a quenching solution containing tripolyphosphate (TPP) to cause an ionic cross-linking reaction between the outer layer of chitosan and TPP. Factors influencing the properties of the capsule membrane, such as the molecular weight of chitosan, the dipping solution and dipping time, and the quenching solution and time, were optimized to successfully produce osmotic chitosan capsules with asymmetric membrane using chitosans that possessed different viscosities. In situ formation of a delivery orifice on the asymmetric membrane of the chitosan capsule was proven by the observation of a jet stream of chlorophyll being released from the capsule. Drugs with different solubility were selected, and a linear correlation between drug solubility and the initial drug release rate calculated from the slope of the drug release profile was used to verify that the delivery orifices that were in situ formed on the asymmetric membrane of the chitosan capsules induced by osmotic effect was responsible for the drug release. Water permeability across the optimally produced asymmetric membrane of the capsule from chitosan of 500 cps (300–700 cps) quenched with TPP for 30 min (C500/TPP30) was determined to be $1.40 \times 10^{-6} \text{ cm}^2 \text{ h}^{-1} \text{ atm}^{-1}$ at $37.0 \pm 0.5^\circ\text{C}$. The encapsulation of poorly water-soluble drugs, felodipine (FE) and nifedipine (NF), in such an asymmetric chitosan capsule was capable of creating a sufficient osmotic effect to activate the release of the drug with the addition of SLS and HPMC. The multiple regression equations of maximal release percent at 24 h for FE and NF confirmed that both sodium lauryl sulfate (SLS) and hydroxypropyl methylcellulose (HPMC) positively influenced this response factor, and the effect of SLS was greater than that of HPMC.

© 2006 Elsevier B.V. All rights reserved.

Keywords: Chitosan; Asymmetric membrane; Osmotic; In situ orifice formation; Poorly soluble drugs

1. Introduction

One advantage of osmotic capsules with asymmetrical membranes is the higher rate of water influx, allowing the release of drugs with lower osmotic pressures or lower solubility (Herbig et al., 1995). Another advantageous characteristic of asymmetrical membranes in osmotic capsules is that the membrane consists of a very thin, dense skin structure supported by a thicker, porous structural layer, and in situ formation of delivery orifices on this thin layer is potentially possible with no assistance (Verma et al., 2002). Previously, the possibility of

forming delivery orifices in situ on asymmetrically coated membranes using cellulose acetate was explored (Lin and Ho, 2003). In situ formation of a delivery orifice on the thin membrane was proven by visualization of a jet stream of chlorophyll being released from the capsule. The release mechanism for drugs with moderate to high water solubility was mainly controlled by the osmotic effect, which is a function of the drug's solubility. Furthermore, two poorly water-soluble drugs, felodipine (FE) and nifedipine (NF), have been utilized to demonstrate how the controlled-release characteristics can be manipulated by the design of polymeric capsules with an asymmetrical membrane and core formulations (Wang et al., 2005). Searching for membrane materials suitable for the construction of osmotic capsules with thin, dense semi-permeable membranes which are potentially able to form a delivery orifice in situ has practical

* Corresponding author. Tel.: +886 2 23771942; fax: +886 2 23771942.
E-mail address: mingsheu@tmu.edu.tw (M.-T. Sheu).

value in terms of facilitating the advancement of this technology.

Chitosan is a cationic polysaccharide derived from the natural polymer, chitin, which is a biocompatible and biodegradable material. Chitosan has been used as a coating material in pharmaceuticals, a carrier for the immobilization of enzymes, and a gel for the entrapment of cells or organisms (Overgaard et al., 1991; Hugnat et al., 1994; Yoshioka et al., 1990; Shinonaga et al., 1992). The design of chitosan hard capsules used for colon therapy was described in patents by the Alcello Co. in 1994 (Suzuki et al., 1994). Tozaki and coworkers examined the acceleration of the healing effect of a new thromboxane synthase inhibitor with chitosan capsules coated with hydroxypropyl methylcellulose phthalate (HPMCP) as an enteric coating material to achieve colon-specific drug delivery (Tozaki et al., 1999). Lim in US patent no. 4,352,883 disclosed a two-stage encapsulation of various biological materials by precipitating sodium alginate with calcium ions and then depositing an anionic–cationic membrane with a polyanion (polylysine) on the surface (Lim, 1982). Rha and Rodriguez-Sanchez in US Patent no. 4,744,933 described the direct formation of capsules through a process of mixing one solution containing an anionic polymer (alginate) and the other containing a cationic polymer (chitosan) (Rha and Rodriguez-Sanchez, 1988). The permeability of the walls of chitosan alginate capsules was reported by Daly et al. to be adjustable by substituting a predetermined fraction of esterified alginic acid for a part of the metal alginate normally used in the fabrication of such capsules (Daly et al., 1989). Mi et al. reported alginate as an anionic polyelectrolyte which can be used to control the swelling and erosion rates of chitosan tablets by anionic–cationic interpolymeric complexation with chitosan (Mi et al., 1997). The preparation and characterization of interpolymeric complexation of chitosan (cation) with chondroitin sulfate (anion) as an encapsulating material was reported by Chen and Wang (2002). However, all those reports did not disclose the potential of forming asymmetric structure on chitosan membrane to induce enough osmotic effects and in situ formation of delivery orifice for activating the drug release, especially for poorly water-soluble drugs.

In comparison to polyanions, the use of low-molecular-weight anions to crosslink chitosan was found to be much simpler and milder. Shu and Zhu investigated the electrostatic interactions between multivalent phosphates (phosphate (Phos), pyrophosphate (Pyro), and tripolyphosphate (TPP)) and chitosan, as well as the influence of electrostatic interactions on the properties of chitosan films ionically crosslinked by the above-mentioned phosphates (Shu and Zhu, 2002). It was concluded that the interaction between chitosan and TPP still existed even in solutions with a pH of <0.5 as a result of a greater number of negative charges of TPP. Because of this, chitosan/TPP exhibited much less pH-sensitive swelling and drug controlled-release properties due to the relatively strong interactions. Furthermore, the chitosan microspheres prepared by ionic coagulation in an aqueous TPP solution with a pH value of <6 resulted in a dense top layer, whereas an open porous structure was observed on the surface when the coagulation proceeded in an aqueous TPP solution of pH 8.9 (Overgaard et al., 1991). The latter results

suggest that the gelation mechanism of chitosan microspheres is actually controlled by the ionic crosslinking reaction between protonated chitosan and ionized TPP leading to the formation of a dense top layer of freeze-dried chitosan microspheres.

In a view to the possibility of forming thin, dense structures using chitosan ionically crosslinked with TPP at a pH value of <6, we attempted to prepare crosslinked chitosan capsules with tripolyphosphate (TPP) to form asymmetric characteristics for activating the osmotic effect and to demonstrate whether delivery orifices could be formed in situ on the membrane in this study. Chitosans with various viscosities were selected and an acetic aqueous/alcoholic solution was used as the solvent. The crosslinking effects of phosphate (Phos) and pyrophosphate (Pyro) solutions were compared as well under similar pH values of <6.

2. Experimental section

2.1. Materials

Chitosans of various grades of viscosity measured at a concentration of 5 g L⁻¹ and 20 °C (10: 5–20 cps; 100: 50–150 cps; 500: 300–700 cps; and 1000: 800–1500 cps), designated C10, C100, C500, and C1000, respectively, were purchased from Wako (Japan). Three chitosans with different molecular weights indicated by the viscosity measured at 1% in a 1% acetic solution at 20 °C (low: ~100 mPa s; medium: ~200 mPa s; and high molecular weight: ~400 mPa s) designated CLmw, CMMw, and CHmw, respectively, and three chitosans with different viscosities measured at 1% in a 1% acetic solution at 20 °C (low viscosity: ≤200 mPa s; medium viscosity: 200–400 mPa s; and high viscosity: ≥400 mPa s), designated as CLvis, CMvis, and CHvis, respectively, were supplied by Fluka (Switzerland). Nifedipine (NF) was provided by Merck (Darmstadt, Germany). Felodipine, chlorpheniramine maleate, pyridoxine, naproxen sodium, diltiazem HCl, chlorophyll, Tween 80, sodium lauryl sulfate (SLS), sodium tripolyphosphate (Na₅P₃O₁₀, TPP) were from Sigma (St Louis, MO, USA). Sodium pyrophosphate (Na₄P₂O₇, Pyro) was purchased from RDH (Seelze, Germany) and sodium phosphate (Na₃PO₄, Phos) was supplied by Mallinckrodt (USA). Hydroxypropyl methylcellulose (HPMC, 5 cps) was from Shin-Etsu (Japan).

2.2. Characterization of chitosan

2.2.1. Molecular weight determination by size exclusion chromatography (SEC)

Determination of the molecular weight and the polydispersity of chitosans from various sources was performed by size exclusion chromatography (SEC) employing a triple detector (ViscoTek TriSEC, model 270 dual detector including a viscometer and light scattering and refractometer (RI detector, ERC-7512, ERC) (Keary, 2001). A TSK Gel column (GMPW_{XL}, 7.8 × 30.0 mm, TOSOH) loaded with 0.1% (w/v) of chitosan samples was eluted with a mobile phase containing 0.3 M acetic acid (AcOH) and 0.2 M sodium acetate (AcONa), pH 4.3, at a flow rate of 0.8 mL min⁻¹ with the column temper-

Table 1
Characterizations of chitosans by SEC with triple detector (TriSEC)

	M_w	M_n	Pd	IV_w (dl/g)	R_{gw} (nm)	R_{hw} (nm)	Viscosity ^a
C10	89800	33940	2.646	1.673	15.82	12.15	5–20 ^b
C100	233800	106400	2.197	5.502	33.29	25.58	50–150 ^b
C500	317500	192600	1.648	10.060	46.32	35.60	300–700 ^b
C1000	476500	314800	1.514	15.377	61.47	47.26	800–1500 ^b
CLmw	112300	61640	1.822	4.171	24.25	18.63	~100 ^c
CMmw	291900	202300	1.443	10.047	45.47	34.95	~200 ^c
CHmw	383000	246400	1.554	10.946	50.80	39.05	~400 ^c
CLvis	108300	41970	2.580	6.225	26.44	20.31	≤200 ^c
CMvis	260700	172200	1.514	10.592	44.31	34.06	200–400 ^c
CHvis	378200	207400	1.864	11.962	51.64	39.69	≥400 ^c

M_w : weight-average molecular weight; M_n : number-average molecular weight; Pd: polydispersity; IV_w : weight-average intrinsic viscosity; R_{gw} : weight-average of radius of gyration; R_{hw} : weight-average of hydrodynamic radius.

^a Viscosity reported by the manufacturer.

^b Units: cp.

^c Units: mPa s.

ature maintained at 35 °C. Elution times and volume markers (pullulan with a weight-average molecular weight which ranged 4.0–8.0 × 10⁵) were determined and used as the calibrator to characterize the molecular weight and polydispersity of the chitosans examined in this study. Results are listed in Table 1.

2.2.2. Viscosity

Chitosan samples were dissolved in an acetic aqueous solution or acetic alcoholic solution at different concentrations, followed by stirring or heating for homogenization. The viscosity was measured using a DV-II+ viscometer (Brookfield Engineering Laboratories).

2.2.3. Degree of deacetylation (DD)

The titration method reported by Chen and Hwa (1996) was followed for the determination of DD values. Chitosan samples (0.5 g) freeze-dried for 24 h were dissolved in 99.50 g of 5% (v/v) acetic aqueous acid. One gram of a chitosan acetic aqueous solution was diluted with 30 mL deionized water. After adding 2–3 drops of 0.1 mL toluidine (indicator), the solutions were titrated with an N/400 PVS-K (potassium polyvinyl sulfate solution) solution. Titration ended as the solution color turned from blue to pink. The degree of deacetylation (DD) was calculated as follows:

$$DD(\%) = \left[\frac{X/161}{(X/161 + Y/203)} \right] \times 100;$$

$$X = \frac{1}{400} \times \frac{1}{1000} \times f \times 161 \times V; \quad Y = 0.5 \times \frac{1}{100} - X;$$

where V is the titrated volume (mL) of N/400 P.V.S.-K solution, and f is the factor of the N/400 P.V.S.-K solution.

2.3. Chitosan capsules

Chitosan capsules were produced using a dip-coating process. Chitosan solutions (w/w, C10: 11.0%; C100: 6.0%; C500: 3.5%; and C1000: 3.0%) were prepared by dissolving chitosan at corresponding concentration in a 3% v/v acetic aqueous solution, and then the above solutions were sonicated and allowed

to stand until the trapped air bubbles disappear. The stainless steel mold pins were dipped into the coating solution. The pins were withdrawn while rotating and allowed to air-dry. Then, the capsules were stripped off the pins, trimmed to size, and kept in desiccators until use. Chitosan capsules with asymmetric membranes were also produced using a dip-coating process. The stainless steel mold pins were dipped into capsule solutions as described above followed by quenching in an optimized TPP (0.5% w/w in 0.05 N NaOH), Pyro (0.75% w/w in 0.05 N NaOH), or Phos (1.0% w/w in 0.05 N NaOH) solution containing 1% w/w glycerin.

After quenching for either 15 or 30 min at room temperature, the pins were withdrawn and allowed to air-dry. Then, the capsules were stripped off the pins, trimmed to size, and kept in desiccators until use.

2.4. Solubility

The solubility was determined by dispersing excess model drugs, including chlorpheniramine maleate (CH), diltiazem HCl (DI), naproxen (NA), pyridoxine (PY), and felodipine (FE), in deionized water maintained at 37.0 °C for at least 72 h with intermittent shaking. Immediately after filtration through a syringe with an inserted filter, the filtrate in the middle portion was sampled and properly diluted. The drug concentration was assayed with a validated method to determine the solubility in deionized water at 37.0 °C. The solubility of the model drugs of CH, DI, NA, PY, and FE was determined to be 576.72 ± 16.24, 593.44 ± 1.78, 385.55 ± 3.66, 224.47 ± 0.96, and 2.80 ± 0.33 μg mL⁻¹, respectively.

2.5. Assay validation of model drugs

Drug concentrations were analyzed by a UV-spectrophotometric method (UV spectrophotometer, model V-550 JASCO, Japan) at a specific wavelength for each model drug (CH, 244 nm; DI, 259 nm; NA, 271 nm; PY, 289 nm; FE, 360 nm; and NF, 350 nm). Recoveries were evaluated by comparing the calculated and measured concentrations. Assay methods were

Table 2
Experimental values of core formulation variables and maximal release % at 24 h for felodipine and nifedipine

Formulation No.	Independent variables (mg)			Dependent variables Maximal released (%) (FE; NF) (Y)
	Felodipine or nifedipine (X ₁)	HPMC 50 cps (X ₂)	SLS (X ₃)	
1	1.60	100.00	100.0	70.8; 66.0
2	18.40	100.00	100.0	53.4; 49.0
3	10.0	100.0	100.0	62.5; 57.0
4	10.0	100.0	16.0	32.1; 30.0
5	10.0	100.0	184.0	84.3; 78.6
6	10.0	16.0	100.0	49.0; 45.0
7	10.0	184.0	100.0	80.2; 69.7
8	5.0	50.0	50.0	47.9; 44.0
9	5.0	150.0	50.0	58.2; 54.0
10	5.0	50.0	150.0	66.0; 63.0
11	5.0	150.0	150.0	82.2; 74.4
12	15.0	50.0	50.0	41.2; 39.0
13	15.0	150.0	50.0	50.5; 48.0
14	15.0	50.0	150.0	53.5; 48.0
15	15.0	150.0	150.0	72.3; 67.0
16	3.75	37.5	37.5	43.0; 40.0
17	7.50	75.0	75.0	53.0; 50.0
18	11.25	112.5	112.5	65.0; 62.0
19	12.50	125.0	125.0	66.6; 60.0
20	18.75	187.5	187.5	79.6; 75.0

validated using the precision and the accuracy of intra- and inter-day assays. Results demonstrated that both the precision and accuracy of inter- and intra-day assays for all model drugs were within the acceptable range of <5%.

2.6. Measurement and analysis of the *in vitro* dissolution rate

The *in vitro* dissolution rate was evaluated using dissolution methodology (Apparatus II, 50 rpm, 37.0 °C, 500–1000 mL of medium with sinker) (model DT-610, JASCO). In all cases, an appropriate volume of sample was withdrawn at predetermined time intervals and assayed by a validated UV absorbance measurement with reference to a calibration curve for each model drug. The release rate was determined from the initial portion for each profile. As indicated by the following equation as reported by Lin et al. (Lin and Ho, 2003), a plot of the release rate versus S^2/MW (where S is the drug solubility and MW is the molecular weight of the drug) should be linear with a slope given by the expression in parentheses (A is the surface area of the capsule, h the wall thickness, L_p is the filtration coefficient, σ is the reflection coefficient, and $\Delta\pi$ is the osmotic pressure difference across the wall). Based on this relationship, the water permeability (L_p) of the semi-permeable membrane of capsule wall was calculated by

$$\frac{dM}{dt} = \left(\frac{A}{h} L_p \sigma RT \right) \frac{S^2}{M.W.}$$

2.7. Osmotic capsule preparations

Chitosan capsules (C500 quenched with TPP for 30 min) were fabricated and filled by hand with the desired amount of

a drug-excipient mixture as designated in Table 2. Both HPMC and SLS were dried and the portions that passed an 80-mesh sieve were collected. Physical mixtures were prepared simply by mixing drugs (FE or NF), HPMC, and SLS with shaking by hand in a plastic bag for at least 10 min. After filling, the capsules were capped and sealed with a sealing solution, which contained chitosan in an acetic aqueous solution. The release of the two model drugs from all formulations tested was determined in 900 mL of medium (with the addition of 1% Tween 80) using the USP paddle method. An average of three replicates for the maximal percentage release at 24 h was reported and used for regression analysis in the Design Expert program.

3. Results and discussion

Characterizations of the molecular weight of chitosans from different sources as determined by SEC with Triple detector (TriSEC) are shown in Table 1. Results indicated that the average molecular weights of chitosans from different sources closely matched the increasing viscosity as indicated by the manufacturer. Two series of chitosan supplied by the same company (Fluka), differentiated by information on either molecular weight or viscosity as provided by the manufacturer, possessed similar molecular properties. Therefore, one of the two series of chitosan with different molecular weights including CLmw, CMmw, and CHmw was selected with C10, C100, C500, and C1000 as the materials that were prepared for the viscosity test in two kinds of solvent mixture; results are plotted in Fig. 1. They indicated that as the molecular weight of chitosan increased, the viscosity also increased correspondingly. In addition, as the ratio of alcohol increased, the viscosity of the chitosan acetic alcoholic solution also increased. C10, C100, C500, and C1000 were eventually selected as the materials we used to attempt

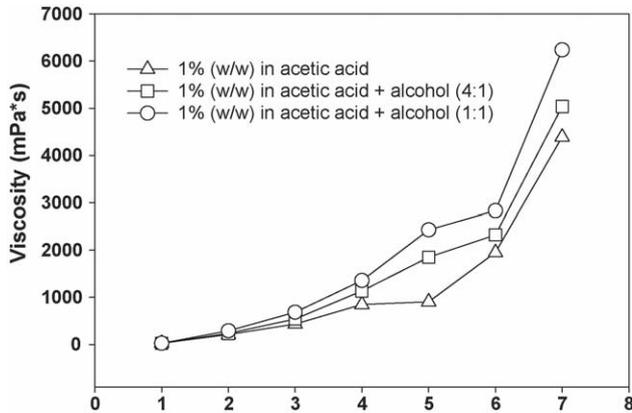


Fig. 1. Viscosity of chitosan solutions (1%) dissolved in 1% acetic acid in either an aqueous solution or alcoholic aqueous solution at a water/alcohol ratio of 4/1 or 1/1. (1, C10; 2, C100; 3, CLmw; 4, CMmw; 5, CHmw; 6, C500; 7, C1000).

to produce chitosan capsules with asymmetric membranes. The DD of those chitosans of C10, C100, C500, and C1000 were found to be 84.3%, 85.6%, 87.7%, and 89.5%, respectively. The results revealed that all DD values were about 85%. Since chitosans with higher DD values have higher amounts of free amino

Table 3

The pH change on the quenched surface of chitosan capsule with respective to time as exemplified by chitosan 500 with TPP

Chitosans	Quenching time			
	0	15 min	30 min	1 h
C10 (11% w/w)	5.42 (13600) ^a	5.99	6.42	6.67
C100 (6% w/w)	4.25 (13780)	5.64	5.78	6.03
C500 (3.5% w/w)	4.32 (13620)	5.70	5.96	6.11
C1000 (3.0% w/w)	4.29 (14000)	5.58	5.91	6.07

^a Figures in parenthesis indicate the initial viscosity of the solution (cp).

groups, it was expected that chitosans with different MWs at the similar DD values would express various strengths for interacting with polyanions including Phos, Pyro, and TPP leading to differences in the extent of ionic crosslinking.

Considering the adhesion of chitosan gels on the stainless steel pins, the coating solution must have suitable viscosity and be easily homogenized. Therefore, the concentration of chitosan in the acetic acid solution was properly determined to have similar viscosities for each of this series of chitosans (C10, C100, C500, and C1000), and the results are listed in Table 2. It was concluded that at the concentration designated, a viscosity of about 13,800 cp seems to be optimal for those four grades

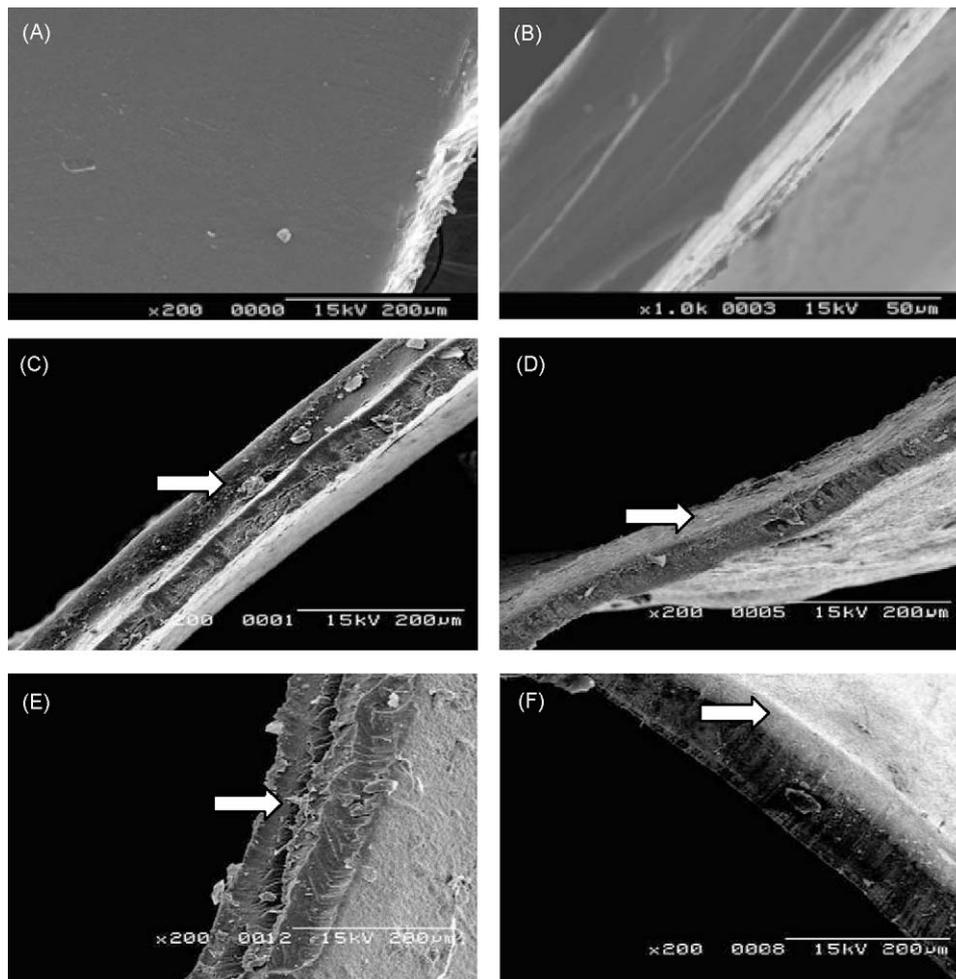


Fig. 2. Scanning electron micrographs of capsule membranes using C500 without quenching. (A) Outer surface at 200 \times magnification; (B) cross-section at 1000 \times magnification; and the cross-section for chitosan capsules prepared with C10 (C), C100 (D), C500 (E), and C1000 (F) quenched with TPP for 15 min.

of chitosan for use in producing capsules. As the results further indicated, C10 and C100 could easily be dissolved in the acetic aqueous solution but required a much greater amount for reaching a suitable viscosity, whereas C500 and C1000 achieved suitable viscosities with lesser amounts but needed a higher concentration of acetic acid in the aqueous solution and a much longer time to completely dissolve. From Table 3, it can further be seen that as the quenching time increased, the pH value increased but only gradually. When the quenching time was increased to 30 min, the pH value on most of the surface still remained at <6 except that for C10. Since the pH value was maintained at a pH value of <6 , the gelation mechanism of chitosan was controlled by actual ionic crosslinking reactions between the protonated chitosan and the ionized tripolyphosphate leading to the formation of a dense layer as part of asymmetric structure (Shu and Zhu, 2002). This was confirmed by examining the surface and cross-section of capsule membranes with SEM photographs.

Fig. 2 illustrates the scanning electron micrographs (SEMs) of the surface (Fig. 2A) and cross-section (Fig. 2B) of the capsule walls for those capsules produced with C500 without quenching as a typical example. It indicates that a smooth membrane

surface was produced using C500 without quenching with TPP. The cross-sectional view of such a chitosan capsule wall demonstrated it to be homogeneous with a thickness of about $50\ \mu\text{m}$. Fig. 2 further shows the cross-sectional view of those capsule wall membranes produced with C10 (Fig. 2C), C100 (Fig. 2D), C500 (Fig. 2E), and C1000 (Fig. 2F) quenched with TPP for 15 min. Clearly, it can be observed that all those membranes were divided into two distinct layers with nearly equal thickness. SEMs in Fig. 3 also show similar asymmetric two-layer structure on the membrane for those chitosan capsules produced with C500 quenched with TPP (Fig. 3A and B), Phos (Fig. 3C and D), or Pyro (Fig. 3E and F) for 15 min (Fig. 3A, C, E) or 30 min (Fig. 3B, D, F). Both weights and dimensions of chitosan capsules were demonstrated to be consistent (data not shown), confirming that the process of producing these capsules is reproducible.

In situ formation of a delivery orifice on the asymmetric membrane for releasing drugs was proven with photographs as shown in Fig. 4. A deeply colored jet stream of chlorophyll from an open hole can be observed when an asymmetric membrane-formed chitosan capsule encapsulating chlorophyll was suspended in the water medium. This delivery process continued for 30, 60, and 180 min as demonstrated by Fig. 4A–C. However, when this

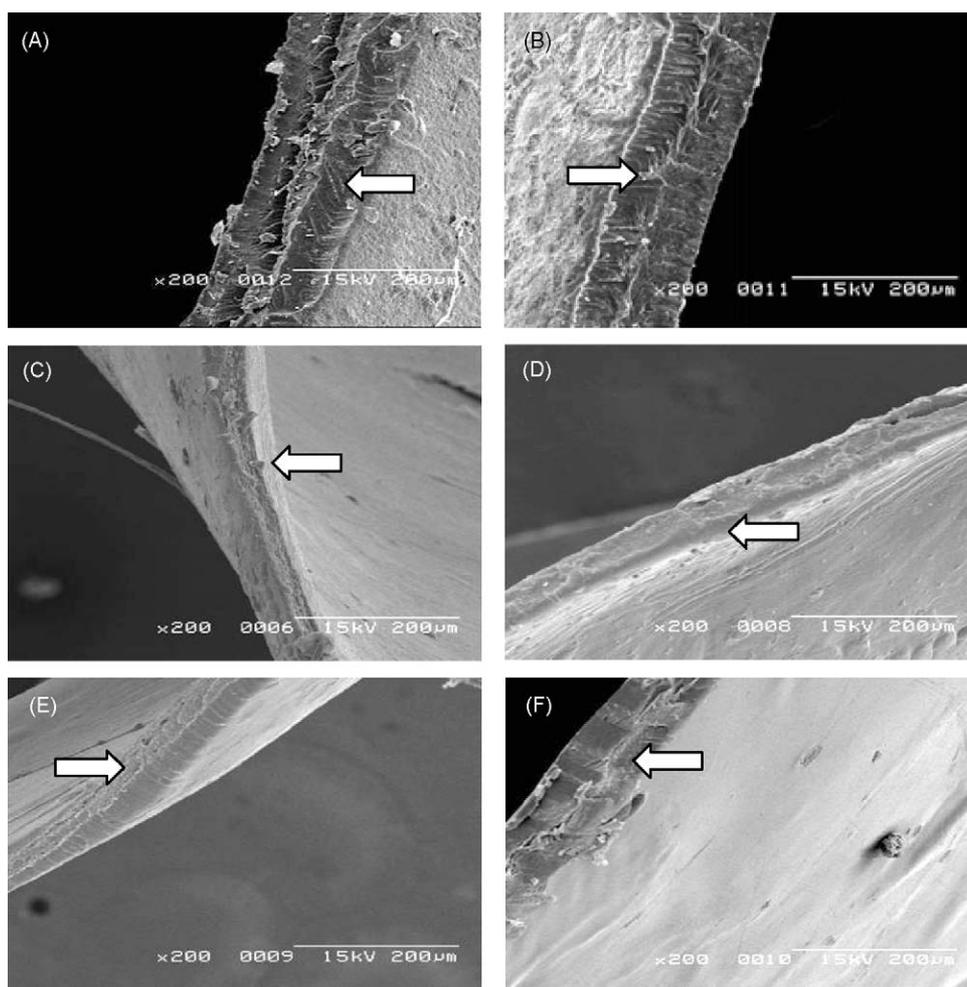


Fig. 3. Scanning electron micrographs of the cross-section at $200\times$ magnification of chitosan capsules using C500 quenched with different polyanions (TPP, A and B; Phos, C and D; Pyro, E and F) for 15 (A, C, and E) or 30 min (B; D, and F).

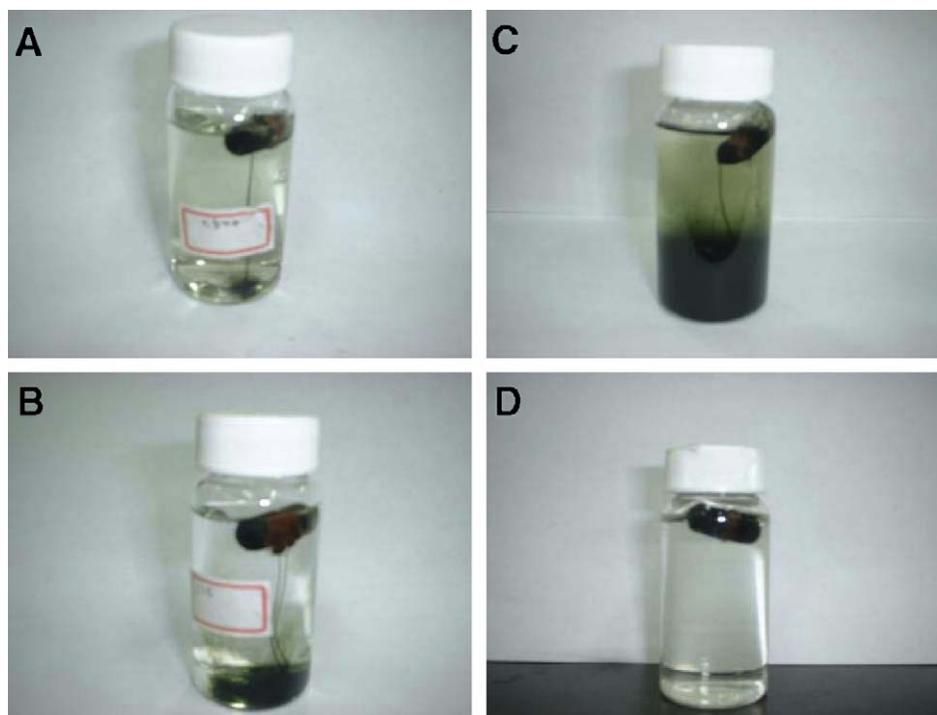


Fig. 4. Photographs of chitosan capsules (C500/TPP30) filled with chlorophyll in water after 30 min (A), 60 min (B), and 3 h (C), or in a NaCl (6% w/w) solution for 1 day (D).

capsule was suspended in a 6% (w/w) NaCl solution, the osmotic effect was inactivated, and no orifices were in situ formed to release chlorophyll (Fig. 4D). This indicates that the in situ formation of a delivery orifice is possible on the thin, dense structure of the asymmetric two-layer membrane of those chitosan capsules. The osmotic pressure created across chitosan membrane to cause the formation of orifice on asymmetric structure might play an important role in the switching on or off of this mechanism.

By using model drugs of various solubilities (CH, DI, NA, PY, and FE), the osmotically controlled drug release from chitosan capsules was further characterized based on the equation in the “Experimental section”. The core formulation consisted of model drugs alone but with varying solubilities in water. The *in vitro* drug release profiles of model drugs from chitosan capsules with asymmetric membrane produced with chitosans of different viscosities (C10, C100, C500, and C1000 cps) and quenched with TPP for 0, 15, or 30 min were examined, and the release profiles from those chitosan capsules are shown in Fig. 5. The *in vitro* profiles of drugs released from chitosan capsules without quenching to form an asymmetric dense structure were instantly measurable and were shown to be very similar even for model drugs with different solubilities in water. This implies that there was no control characteristic of the drug release for those chitosan capsules without ionic crosslinking with those polyanions examined in this study. Furthermore, release rates decreased with increasing quenching times, but there were no significant differences among chitosan capsules prepared with different viscosity grades of chitosan (data not shown). This can be explained by the fact that the drugs were not released from

chitosan capsules by diffusion but through an orifice created by the osmotic pressure on asymmetric membrane of capsule wall. Therefore, although the permeability of drugs through the membrane was expected to differ for those capsules produced with different viscosity grades of chitosan (different molecular weights and concentrations), the permeation of drugs through the membrane was not responsible for the release mechanism from those capsules produced with different viscosity grades of chitosan leading to the conclusion that asymmetric chitosan membranes so formed release drugs via the orifice created by osmotic pressure.

The *in vitro* drug release profiles of capsules formulated using C500 quenched with TPP for either 15 (C500/TPP15) or 30 min (C500/TPP30) are shown in Fig. 6A and B, respectively. The initial portion of the drugs release profiles was used to calculate the initial drug release rate for the model drugs from the chitosan capsules. The results revealed that both the amount released and the release rate were related to drug solubility. The amount released was larger and the drug release rate was faster as the drug solubility increased. Obviously, the erosion phenomenon of chitosan capsules was observable for those capsules produced without quenching (data not shown). During the quenching process, chitosan and TPP were cross-linked forming a dense structure as part of asymmetric membrane for the osmotic control of drug release. The quenching time enhanced the membrane strength by increasing the extent of crosslinking, and it also reduced the erosion of chitosan. Moreover, the asymmetric two-layer structure was obvious as illustrated in Figs. 2 and 3. The dense layer was expected to be the major factor controlling the release of drug from the capsules. How-

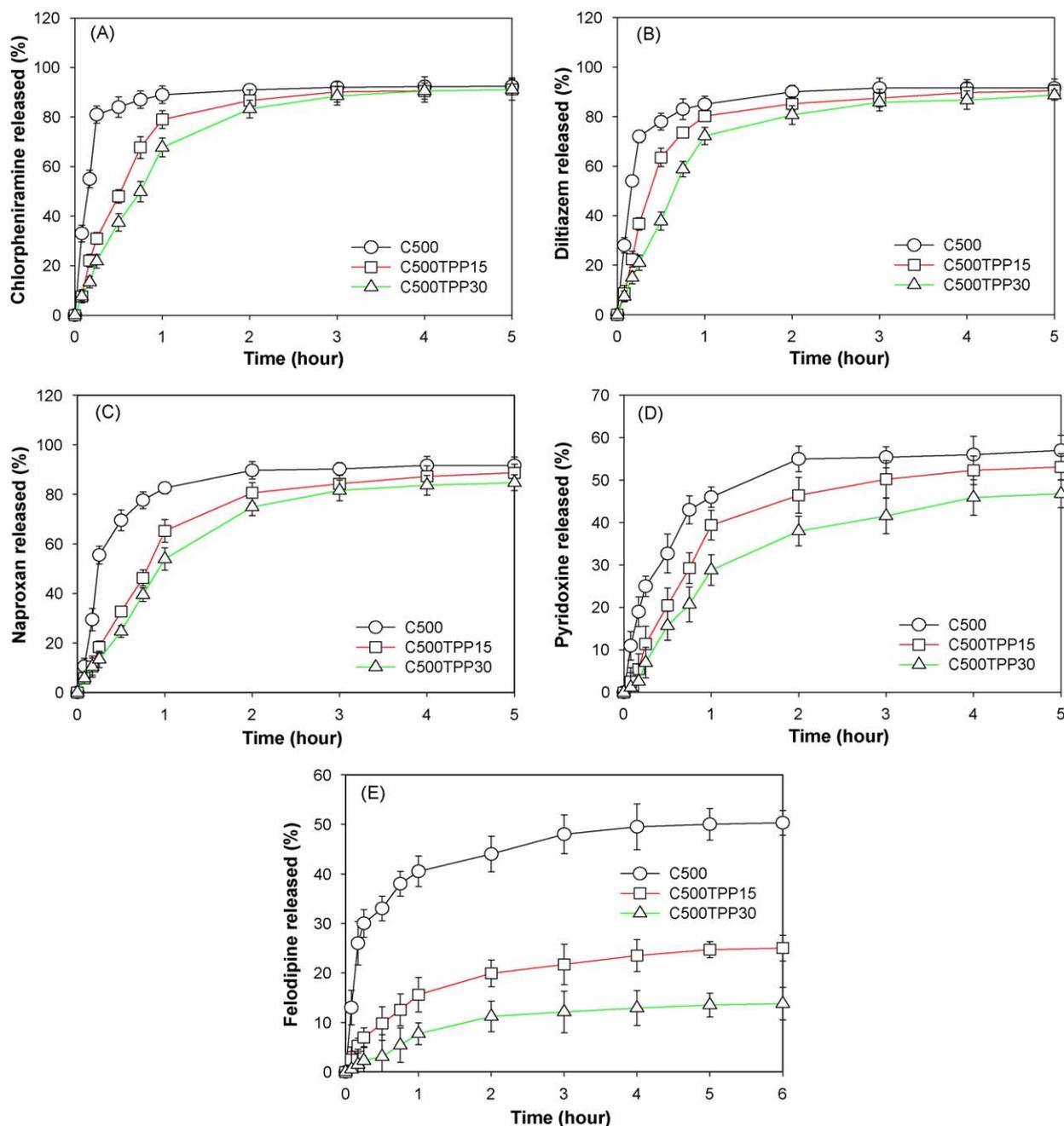


Fig. 5. The release profiles of five model drugs of chlorpheniramine (A), diltiazem (B), naproxen (C), pyridoxine (D), and felodipine (E) from chitosan capsule formulations of chitosan 500 quenched for either 0 (○), 15 (□), or 30 min (△).

ever, it was observed that this dense layer did not differ between that quenched for 15 min and that for 30 min.

The graphs displayed in Fig. 6C and D plot the release rate (dM/dt) of model drugs from C500/TPP15 and C500/TPP30 capsules, respectively, versus the ratio of the square of the drug solubility to molecular weight (S^2/Mw) based on the equation described. A linear correlation between the initial drug release rate (calculated from the slope of the drug release profile) and S^2/Mw was observed. The slope of the linear portion was $0.0401 \text{ h}^{-1} \text{ M}^{-1}$ for the capsule formulation of C500/TPP15 and $0.0397 \text{ h}^{-1} \text{ M}^{-1}$ for that of C500/TPP30 when keeping

all other factors constant. A statistically significant correlation was demonstrated for both ($r^2 = 0.9936$ (C500/TPP15) and 0.9913 (C500/TPP30)). This is consistent with drug release by an osmotic pumping mechanism. Assuming ideality, where R is the universal gas constant and T is the absolute temperature, L_p (at 37.0°C) was calculated to be 1.56×10^{-6} and $1.40 \times 10^{-6} \text{ cm}^2 \text{ h}^{-1} \text{ atm}^{-1}$ for C500/TPP15 and C500/TPP30, respectively, with known values of h and A . As concluded for asymmetric membrane-coated capsules with in situ-formed delivery orifices, the osmotic effect to induce the in situ formation of orifice is the main activation force for drug release.

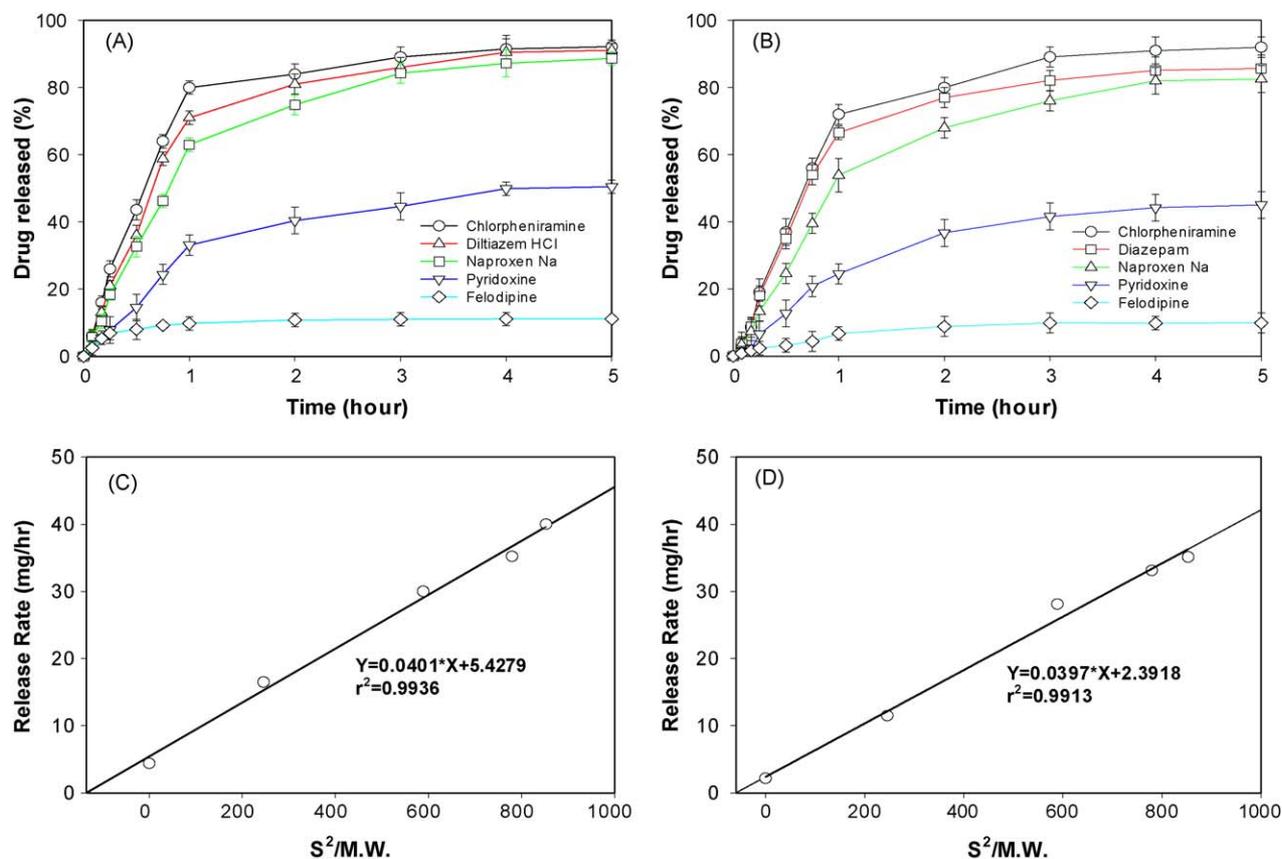


Fig. 6. The release profiles of model drugs from chitosan capsules (A, C500/TPP15; B, C500/TPP30) and the linear relationship between the release rate and the square of the solubility divided by the molecular weight of model drugs for chitosan capsules (C, C500/TPP15; D, C500/TPP30).

Similarly, drug solubility is responsible for the successful engineering of the thin, dense asymmetric membrane-coated capsules with an in situ-formed delivery orifice to release drugs at a desirable rate.

Furthermore, the water permeability ($\times 10^{-6} \text{ cm}^2 \text{ h}^{-1} \text{ atm}^{-1}$) for C500 capsules quenched with two other multivalent phosphates for 15 (Phos15, Pyro15, and TPP15) or 30 min (Phos30, Pyro30, and TPP30) was determined. Results revealed that there was a significant difference between those capsules quenched for 15 and 30 min with Phos, but differences between those quenched for 15 and 30 min with Pyro or TPP were minimal (C500/Phos15: 0.82×10^{-6} ; C500/Phos30: 1.27×10^{-6} ; C500/Pyro15: 0.96×10^{-6} ; C500/Pyro30: 0.99×10^{-6} ; C500/TPP15: 1.56×10^{-6} ; and C500/TPP30: $1.40 \times 10^{-6} \text{ cm}^2 \text{ h}^{-1} \text{ atm}^{-1}$). Permeability effects seemed to mainly be influenced by quenching times and viscosity grades of the chitosan used. Although capsules treated with quenching for 15 min seemed to be better, they were much more difficult to manufacture. A sufficient time was needed to promote the crosslinking effect and strengthen the chitosan/TPP surface layer to resist the inner swelling force of the chitosan layer. In

comparison with Pyro and Phos, TPP exhibited a better crosslinking effect with chitosan. Therefore, C500 quenched with TPP for 30 min (C500/TPP30) was selected as the model capsule for further study.

An experimental design was conducted to examine the quantitative effects of HPMC and SLS on the maximal release percent at 24 h for two poorly soluble drugs of felodipine (FE) and nifedipine (NF). Table 2 illustrates all formulations designed, and Fig. 7 demonstrates the release profiles of FE and NF from each formulation. To quantify the influence of formulation variables on the maximal release (%) at 24 h from C500/TPP30 chitosan capsules, a multivariable linear regression analysis using the Design Expert program was conducted based on this experimental design. A multivariable linear function in the form of $Y=f(X_i)$ was used as the fitting equation. X_i is the independent variable ($i=1, 2, 3$) representing the FE (or NF) loading, the added amount of HPMC, and the added amount of SLS, respectively, and Y is the dependent variable representing the maximal release (%) of FE (or NF) at 24 h. The regression equations were obtained following a stepwise method for FE and NF, respectively:

$$Y_F = 32.9640 - 1.0654 X_1 + 0.1470 X_2 + 0.2305 X_3 \quad (\text{adj. } R^2 = 0.9197)$$

$$Y_N = 30.9946 - 0.9596 X_1 + 0.1280 X_2 + 0.2132 X_3 \quad (\text{adj. } R^2 = 0.9232)$$

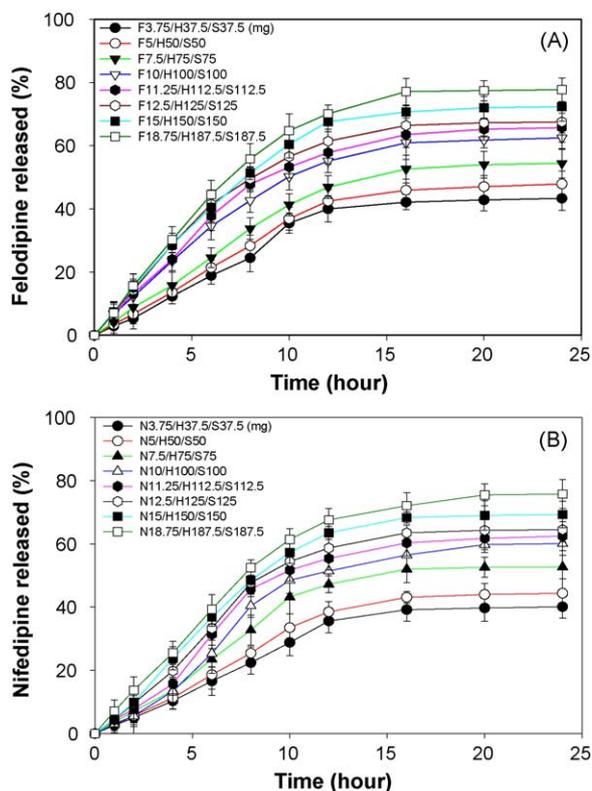


Fig. 7. Representative release profiles of felodipine (A) and nifedipine (B) in 1% Tween 80 solutions from chitosan capsules (C500/TPP30) filled with those formulations containing various amounts of felodipine (F) or nifedipine (N) and a fixed ratio (10 \times) of physically-mixed SLS (S) and HPMC (H) designed by experimental design.

The calculated values by fitting the equation and the experimental values of the maximal percentage released at 24 h were fairly well correlated as shown in Fig. 8A (*adj. R*²=0.9197 for FE) and 8B (*adj. R*²=0.9232 for NF). From the regression results, the following conclusions were reached: (1) drug loading (X_1) had a negative influence on the maximal release percent, whereas both the added amounts of HPMC (X_2) and SLS (X_3) significantly enhanced the maximal release percent at 24 h; (2) the quantitative influence of SLS was greater than that of HPMC; (3) a synergistic interaction of the added amounts of HPMC and SLS (X_2X_3) on the maximal release percent at 24 h was found to be statistically insignificant.

The added amounts of SLS and HPMC both had a positive influence on the release profile. Being an osmotic agent, SLS is able to improve the solubility of FE and NF in deionized water. The sole role that HPMC may play was as a thickening agent to elevate the viscosity of the core suspension for suspending drug particles. As a result, the release rate increased when an increase in the amount of HPMC added by increasing the available surface area of drug particles for dissolution. Because of that, both HPMC and SLS enhanced the release rate of FE and NF, but SLS was superior to HPMC.

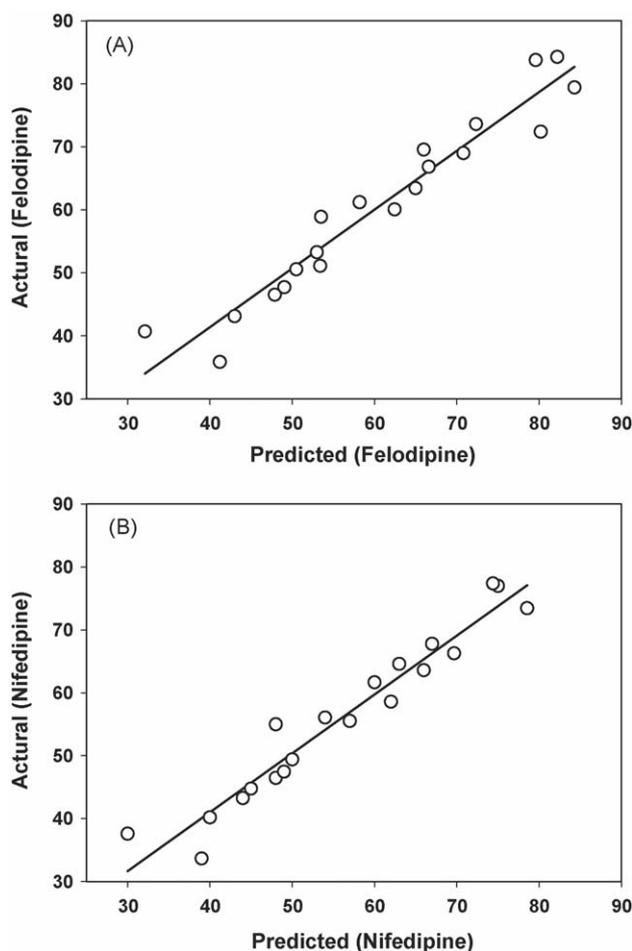


Fig. 8. Correlation of calculated and experimental values of the maximal release (%) of felodipine (A) and nifedipine (B) at 24 h.

4. Conclusions

Chitosan/TPP crosslinked membranes were characterized as being asymmetric with osmotically activated in situ formation of a delivery orifice for releasing drugs in this study. In addition, chitosan/multivalent phosphate membranes also demonstrated the same characteristics as did the chitosan/TPP crosslinked membranes. The in situ-formed delivery orifice on asymmetric membrane of controlled-release polymeric capsules was mainly responsible for the delivery of both soluble and poorly soluble drugs. In vitro release studies indicated that drug delivery from this asymmetric membrane-coated system is principally controlled by osmotic pressure for those drugs with moderate to high water solubility. The thin and dense asymmetric membrane-coated capsules (C500/TPP30) prepared in this study had a permeability of $1.40 \times 10^{-6} \text{ cm}^2 \text{ h}^{-1} \text{ atm}^{-1}$. This parameter can be used to predict the release rate of any drug encapsulated in this chitosan capsule. Solubilization of poorly water-soluble drugs and thickening agents, such as HPMC, mixed with the solubility enhancers are able to increase the release rate and the cumulative released amount.

References

- Chen, R.H., Hwa, H.D., 1996. Effect of molecular weight of chitosan with the same degree of deacetylation on the thermal, mechanical and permeability properties of the prepared membrane. *Carbohydr. Polym.* 29, 353–358.
- Chen, W.M., Wang, L.F., 2002. Proceedings of the 3rd Asian International Symposium on Biomaterials and Drug Delivery Systems, Biomaterials and Controlled Released Society of Taiwan, pp. 399–402.
- Daly, M.M., Keown, R.W., Knorr, D.W., 1989. Chitosan alginate capsules, United States Patent Number 4,808,707.
- Herbig, S.M., Cardinal, J.R., Korsmeyer, R.W., Smith, K.L., 1995. Asymmetric membrane tablet coatings for osmotic drug delivery. *J. Control. Release* 35, 127–136.
- Hugnat, M.L., Groboillot, A., Neufeld, R., Poncel, J., Dellacherie, D.E., 1994. Hemoglobin encapsulation in chitosan/calcium alginate beads. *J. Appl. Polym. Sci.*, 1427–1432.
- Keary, C.M., 2001. Characterization of METHOCEL cellulose ethers by aqueous SEC with multiple detectors. *Carbohydr. Polym.* 45, 293–303.
- Lim, F., 1982. Encapsulation of biological material, United States Patent No. 4,352,883.
- Lin, Y.K., Ho, H.O., 2003. Investigations on the drug releasing mechanism from an asymmetric membrane-coated capsule with an in situ formed delivery orifice. *J. Control. Release* 89, 57–69.
- Mi, F.L., Her, N.L., Kuan, C.Y., Wong, T.B., Shyu, S.S., 1997. Chitosan tablets for controlled release of theophylline: Effect of polymer – drug wet or dry blending and anionic–cationic interpolymer complex. *J. Appl. Polym. Sci.* 66, 2495–2505.
- Overgaard, S., Schärer, J.M., Moo-Young, M., Bols, N.C., 1991. Immobilization of hybridoma cells in chitosan alginate beads. *Can. J. Chem. Eng.* 69, 439–443.
- Rha, C., Rodriguez-Sanchez, D., 1988. Process for encapsulation and encapsulated active material system. United State Patent No. 4,744,933.
- Shinonaga, M.A., Kawamura, Y., Yamane, T., 1992. Immobilization of yeast cells with cross-linked chitosan beads. *J. Ferment. Bioeng.* 74, 90–94.
- Shu, X.Z., Zhu, K.J., 2002. The influence of multivalent phosphate structure on the properties of ionically cross-linked chitosan films for controlled drug release. *Eur. J. Pharm. Biopharm.* 54, 235–243.
- Suzuki, T., Hashiudo, K., Matsumoto, T., Higashide, T., 1994. Large intestinal dissociative hard capsules. United State Patent No. 5,283,064.
- Tozaki, H., Fujita, T., Odoriba, T., 1999. Colon-specific delivery of R68070, a new thromboxane synthase inhibitor, using chitosan capsules: therapeutic effects against 2,4,6-trinitrobenzene sulfonic acid-induced ulcerative colitis in rats. *Life Sci.* 64, 1155–1162.
- Verma, R.K., Krishna, D., Garg, M.S., 2002. Formulation aspects in the development of osmotically controlled oral drug delivery systems. *J. Control. Release* 79, 7–27.
- Wang, C.Y., Ho, H.O., Lin, L.H., Lin, Y.K., Sheu, M.T., 2005. Asymmetric membrane capsules for delivery of poorly water soluble drugs by osmotic effects. *Int. J. Pharm.* 297, 89–97.
- Yoshioka, T., Hirano, R., Shioya, T., Kako, M., 1990. Encapsulation of mammalian cell with chitosan CMC capsule. *Biotechnol. Bioeng.* 35, 66–72.