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Preparation and evaluation of newly developed chitosan salt coating dispersions for colon delivery without requiring overcoating

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Summary

Although chitosan (CS) has been recognized as one of good materials for colon-specific drug delivery systems, an overcoaitng with an enteric coating polymer on the surface of CS is absolutely necessary because CS is soluble in acidic conditions before reaching colon. In the present study, to improve its stability towards acid, a newly developed CS-laurate (CS-LA) material was evaluated as a coating dispersion for the development of colon-specific drug delivery systems. Two types of CS such as CS250 and CS600 which have different molecular weights were used to prepare CS-LA films by the casting method. The CS250-LA films had smooth surfaces, whereas the surfaces of the CS600-LA films were rough, indicating that the CS250-LA dispersion could form a denser film than CS600-LA. Both of the CS-LA films maintained a constant shape over 22 h in a pH 1.2 HCl/NaCl buffer, where the corresponding CS films rapidly disintegrated. In addition, the CS250-LA film showed specific colon degradability in pH 6.0 phosphate buffered solution containing 1.0% (w/v) β-glucosidase. As the results of tensile strength and elongation at break, both CS-LA films were found to have flexible film properties. Finally, the release of acetaminophen from disks coated with CS250-LA dispersions was significantly suppressed in fluids at pH 1.2 and 6.8, whereas the disks coated with CS solutions rapidly released drug in pH 1.2 fluids. Taken together, this study shows that LA modification could be one of useful approaches to prepare CS films with acid stability and colonic degradability properties without requiring overcoating.

Keywords*: *Chitosan derivatives; acid stability; colonic delivery; coating dispersions; film.

***Abbreviations*:** 5-ASA, 5-aminosalicylic acid; CS, chitosan; LA, lauric acid; CS250, medium molecular weight chitosan; CS600, high molecular weight chitosan; CS-LA,

chitosan-laurate; Tween 80, polyoxyethylene (20) sorbitan monooleate; FT-IR, Fourier-transform infrared spectroscopy; SEM, scanning electron microscope; TS, tensile strength; EB, elongation at break; APAP, acetaminophen.

1. Introduction

Interest in the design of oral, colon-specific drug delivery systems has increased considerably during the last decade. [1)] The possibility of being able to deliver drug molecules directly to the diseased part of a colon, such as those parts affected by inflammatory bowel diseases (IBD) and colon cancer, would mean that lower doses of the drugs could be used to achieve therapeutic levels. Furthermore, the side effects typically associated with the systemic absorption of drugs from the upper part of the gastrointestinal tract could be reduced or even eliminated entirely by suppressing the release of the drug until the delivery device enters the colon where a triggering mechanism initiates release. Taken together with the fact that the number of IBD patients is currently increasing, [2)] there is an obvious need for the development of an effective drug delivery system for the colon.

Numerous studies have already been conducted to develop drug delivery systems for the colon. Among them, chitosan (CS) has attracted considerable attention as a good material for the development of colon-specific drug delivery systems. [3)] CS is a natural functional polymer containing 2-amino-2deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose units, which are linked together through β-(1-4) bonds, [4)] and the glycosidic bonds making up the molecular chain in CS can be degraded by *β*-glucosidases secreted from enteric bacteria. Numerous reports have appeared in the literature pertaining to the formulation of CS capsules and tablets containing a variety of different drugs. [5,6)] For example, Macleod et al. reported that tablets coated with a 3:1:1 (w/w/w) combination of pectin, hydroxypropyl methylcellulose polymers and CS remained intact through the stomach and small intestine, and were then specifically disintegrated in the human colon. [6)] Recently, a CS coating solution known as Chitocoat® was marketed as a commercial product, and Furukawa et al. reported that tablets coated with Chitocoat released only 10% of the total drug after they had been suspended in first fluid (JP15) for 2

h or second fluid for 3 h, whereas this Chitocoat coated tablet released 100% of the drug in colonic fluid after 7 h. [7] Furthermore, Furukawa et al. confirmed that the tablets were specifically disintegrated in the colons of healthy adults by X-ray contrast radiography. [8] However, since CS is itself dissolved under acidic conditions because of its amino groups (at C-2), overcoating with an enteric coating polymer such as hydroxypropyl methylcellulose phthalate is absolutely necessary to prevent these CS capsules and CS-coated tablets from disintegrating under acidic conditions for colon-specific drug delivery. This additional procedure increases not only the size of the final pharmaceutical product, but also the level of complexity and overall cost of the manufacturing process. The development of a new strategy to avoid the need for the overcoating of these CS formulations is therefore a matter of significant concern.

One potential strategy to avoid the overcoating process involves the use of CS derivatives whose amino groups (at C-2) have been modified to make them less susceptible to acidic degradation. Several studies have been reported concerning the use of modified CS derivatives, where the release of drug molecules from the corresponding formulations could be suppressed in simulated gastric fluid. For example, Rekha and Sharma reported that lauryl succinyl CS-nano/micro particles including insulin showed only 10% insulin release from these particles when they were treated at pH 1.2 for 2 h, whereas approx. 75% of insulin was released when native CS under the same conditions. [9] In addition, we also prepared the fatty acid salts of CS (CS-laurate and CS-palmitate) and evaluated the release of ranitidine from matrices tablets containing these CS derivatives and the drug. [10] The results of this study revealed that the release of the drug was limited to approximately 30% of the total drug after 2 h in 0.1 M HCl, whereas approximately 90% of the drug was released from the corresponding control tablets containing unmodified CS. These results therefore show that the modification of the amino groups (at C-2) of CS can be used as a

facile strategy to increase the acid stability of CS. However, as for the lauryl succinyl CS described above, this material could only be prepared using methanol or *N*,*N*-dimethylformamide, [9] which are classified as class II solvents in the Guideline for Residual Solvents, and the removal of any residual solvent could therefore cause significant operational issues during the manufacturing and formulation processes. In contrast, CS-LA and CS-palmitate can be readily prepared without the need for harmful solvents. In addition, both lauric acid (LA) and palmitic acid themselves are harmless to humans. Furthermore, since the CS-LA tablets have been reported to show a high increase in water uptake compared with CS-palmitate tablets, [10] CS-LA would be a suitable material for drug delivery into the colon where little water exists. [11] We recently reported that CS-LA also showed a superior lubrication property in tableting process [12]; however, there have been no reports in the literature pertaining to the use of CS-LA in coating process.

With this in mind, the aim of this study was to prepare and evaluate novel CS-LA coating dispersions that exhibited good stability under acidic conditions and specific biodegradability in the colon for colon-specific drug delivery. In fact, we initially prepared CS-LA dispersions and then prepared CS-LA films using a casting method. Two different type of CS with median molecular weights 250 and 600 kDa were used in the current study because it is well known that the characteristics of CS are dependent on their molecular weight. Following a series of analytical experiments to determine the physicochemical properties of films, the optimal dispersions were selected and sprayed directly onto the surface of acetaminophen (APAP) disks to evaluate the drug release from the disks in various testing fluids *in vitro*.

2. Materials and methods

2.1. Materials

Medium molecular weight CS (CS250) (190–310 kDa) and high molecular weight CS (CS600) (600 kDa) with 75–85% deacetylation were purchased from Sigma Aldrich (St. Louis, MO, USA). LA, glycerin and polyoxyethylene (20) sorbitan monooleate (Tween 80) were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Chitocoat was provided as a gift from Freund Co., Ltd (Tokyo, Japan). β-Glucosidase from almonds (β-glucosidase activity : 30 units/mg) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Distilled water was used in all of the experiment, and all of the other chemicals and reagents used in the current study were purchased as the pure laboratory grade.

2.2. Preparation and physicochemical evaluation of CS-LA

2.2.1 Preparation of CS-LA

An excess of CS250 and CS600 was added to acetic acid (0.15% w/v in water), and the resulting mixture was stirred vigorously overnight. The excess solid CS was then removed by centrifugation (Centrifuge 5430; Eppendorf, Hamburg, Germany) at 7830 rpm to give the supernatant as a saturated CS solution. The concentrations of CS250 and CS600 in the respective saturated solutions were found to be 8.68±0.07 and 9.32±0.10 mg/mL using a drying method, respectively (**Table 1**). Sodium LA (1.5% w/w) was prepared by adding LA to a 0.3% (w/v) solution of NaOH in water. Sodium LA (500 mL) was then added to 1000 mL of each CS saturated solution, and the resulting mixtures were stirred vigorously for 1 h at room temperature. Following a 2 h period of incubation, the mixtures were passed through a 420 μm sieve to give the gelled CS-LA, which was washed with distilled water and dried at 55 °C for 72 h. The resulting solid CS-LA samples were then milled on a TI-300 Samplemill (Heiko

Ltd, Tokyo, Japan) at 10,000 rpm followed by an FPS-1 edge mill (Terada seisakusho Ltd., Shizuoka, Japan). A large amount of ethanol, which is a good solvent for LA, was added to the milled CS-LA materials, and the resulting suspensions were filtered to remove any unreacted LA. This filtration process was repeated three times, and the resulting solid was dried at 55 °C for 12 h to remove any residual solvent and then stored in a desiccator overnight. The dried CS-LA material was then milled once again on the edge mill to give a fine powder of CS-LA, which was passed through a 125 μm sieve before being used.

2.2.2. Measurement of the modification pattern of CS-LA using Fourier-transform infrared spectroscopy (FT-IR)

LA was ground into a fine powder using a mortar and pestle prior to analysis. The FT-IR spectra of powdered samples of CS, LA and CS-LA were recorded on a DuraSample IR II system (Shimadzu, Kyoto, Japan) at room temperature using an IRPrestige®-21 (Shimadzu). The FT-IR spectra were recorded under the following conditions: apodization function, Happ-Genzel; range of wavenumber, 500–4000 cm^{-1}; and the number of integration times, 20 times.

2.2.3. Measurement of modification ratio

The modification ratio of LA to the amino groups of CS was measured using a viscometer. The viscosity of the CS solution decreased as it reacted with LA to form CS-LA, and this change was practically applied to determine the extent of the reaction. [10)] An arbitrary amount of 1.5% (w/v) sodium LA was added to a specific amount of the saturated CS solution and the total amount of reaction solution was adjusted by adding an arbitrary amount of distilled water. After the reaction, CS-LA was removed by centrifugation as mentioned above, and the viscosity of supernatant was measured using a TVB-10M viscometer (Toki Sangyo

Co., Ltd., Tokyo, Japan). The modification ratio of LA to the amino groups of CS was calculated from the concentration of sodium LA when the reduction in the viscosity reached its lower limit. The molar concentration of amino groups on CS was calculated from the average molecular weight per CS monomer. This calculation was based on the degree of deacetylation of CS being 80%, because the degree of deacetylation of CS was reported to 75–85% by the supplier.

2.3. Preparation and physicochemical evaluation of CS-LA dispersion

2.3.1. Preparation of CS solutions and CS-LA dispersions

Acetic acid (0.3 g), Tween 80 (0.2 g) and glycerin (1.0, 2.0 or 3.0 g), which was used as a plasticizer, were added to distilled water (160 mL), and the resulting mixture was stirred with a spatula for 5 min. Two grams of CS or CS-LAs was then added to the solution, and the resulting mixture was stirred with a spatula for 5 min. The mixture was then premixed using a Speed Stabilizer (Kinematica, Luzern, Switzerland) at 20,000 rpm for 1 min before being degassed on an ARE-250 planetary centrifugal mixer (Thinky Co., Tokyo, Japan) at 2,200 rpm for 1 min. The premixed suspension (5 mL) was then applied to a probe-sonicator (Sonifier 250; Branson, Danbury, America) with its adjustable output control set at 6 for 5 min. **Table 2** shows the formulation of the dispersion.

2.3.2. Measurement of the diameter of the dispersion

The diameter of the CS-LA particles in dispersion was determined using a Mastersizer2000 laser diffraction particle size analyzer (Marvern, Worcestershire, UK). Ultrasonic treatment was not conducted during the evaluation process and the agitation speed was at 1750 rpm. All of these measurements were performed in triplicate. Relative width of particle size (R_W) was calculated by $(d_{90} - d_{10})/d_{50}$, where d_{10}, d_{50}, and d_{90} are 10%,

50%, and 90% of typical particle size distribution, respectively.

2.4. Preparation and physicochemical evaluation of the polymer films

2.4.1. Preparation of the polymer films

The polymer films were prepared using the casting method. The polymer dispersion or Chitocoat was poured into a petri dish that had been covered with a Teflon seal (TZ-13; Kawada Co., Tokyo, Japan). **Table 3** shows the formulation of Chitocoat. The thickness of the films was adjusted to 100 μm. The films were subsequently cut into a range of different sizes and used as samples.

2.4.2. Surface observation of the polymer films

The morphological characteristics of the surfaces of the films were evaluated by scanning electron microscope (SEM) (JSM-5310LV; JEOL Ltd., Tokyo, Japan). Samples of the films were cut into pieces, placed on double-sided adhesive tape and sputter-coated with platinum under vacuum for 90 seconds prior to being visualized by SEM.

2.4.3. Evaluation of the acid stability of the polymer films

Disintegration tests were conducted using 1st fluid (pH 1.2) as a test fluid at 37 ± 0.5 °C according to the instruction provided by the Japanese Pharmacopoeia 16th edition (JP16). All of the polymer films used in this test were 1 cm^2 in size.

2.4.4. Evaluation of the biodegradability of polymer films in colon

Polymer films (1 cm^2) were added to 10 mL of a phosphate buffered solution (pH 6.0) in a centrifuge tube (50 mL) both in the presence and absence of 1.0% (w/v) *β*-glucosidase, and the resulting mixtures were agitated on a shaker (MMS-210; EYELA,

Tokyo, Japan) at 180 rpm for 24 h in an incubator (FMS-1000; EYELA, Tokyo, Japan) at 37 °C. [13] The films were then washed with distilled water and dried at 55 °C. The weight loss of the films was then calculated using the following equation (Eq. (1)):

$$W_{loss} = \frac{W_i - W_f}{W_i} \times 100 \quad (1)$$

Where W_{loss} is the weight loss of the films after 24 h, W_i is the initial weight of the films and W_f is the final weight of the films after 24 h.

2.4.5. Evaluation of the mechanical properties of the polymer films

A strip of film (1 cm × 4 cm) was fixed at either end to the attachments of a Tensilon precision universal tester (Orientec, Tokyo, Japan), and the film was then pulled apart at a rate of 10 mm/min until it broke. The load (N) and elongation (mm) values at the point that the film broke were detected, and the tensile strength (TS) and elongation at break (EB) were calculated using the experimental data according to Eqs. (2) and (3), respectively, as follows:

$$TS\,(MPa) = \frac{Tensile\ load\,(N)}{Rupture\ area\,(mm^2)} \quad (2)$$

$$EB\,(\%/mm^2) = \frac{Elongation\,(mm)}{Initial\ length\,(mm)} \times \frac{100\,(\%)}{Rupture\ area\,(mm^2)} \quad (3)$$

Where rupture area indicates the area of the fracture surface. In this study, the rupture area was simply regarded as a vertical section of the film, and the TS and EB values were therefore calculated using a rupture area of 100 × film thickness (mm) because all of the films were 1 cm in width.

2. 5. Drug dissolution test from disks coated with CS and the CS-LA polymer

Disks coated with the dispersion were prepared to evaluate the rate of drug release

through the film coatings.

2.5.1. Preparation of the disks

APAP tablets (300 mg, diameter; 13 mm) were prepared using a hydraulic single punch tableting machine at 100 MPa. The disks were prepared by embedding the tablets into polyvinyl chloride pipes (inner diameter 1.33 cm; height 0.7 cm) and using coins and aluminum foil to cover the reverse side of disks.

2.5.2. Preparation of the coating disks

A spray nozzle was fixed 2.5 cm above the disk, and a small amount of Chitocoat or CS250-LA dispersion containing 1.0% (w/w) glycerin was sprayed onto the surface of the disk at 0.75 MPa of spray pressure. The resulting disk was then thoroughly dried using a hair dryer. These operations were repeated until the amount of weight gained by the disk after drying reached 28 mg.

2.5.3. Dissolution test

The dissolution of APAP from the coated disks was examined using the paddle method listed in JP16. The test solution was composed of 900 mL of hydrochloride buffer (pH 1.2) or phosphate buffered solution (pH 6.8) at 37 ± 0.5 °C and a paddle rotation speed of 50 rpm. Samples were removed from the mixture at 5.0, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105 and 120 min. The absorbance values of the samples at 243 nm were determined using a UV-mini spectrophotometer (Shimadzu), and the APAP concentrations were calculated based on the absorbance value of a standard solution.

2.6. Statistics

Statistical analyses were performed using the Student *t*-test. A probability value of $p < 0.05$ was considered to indicate statistical significance.

3. Results and discussion

3.1. Characterization of CS-LA

Figure 1 shows the FT-IR spectra of CS, LA and CS-LA, where the letters A and B have been used to indicate materials based on CS250 and CS600, respectively. All of these spectra contained a typical band in the region of 2850–2930 cm^{-1}, which was attributed to a C–H stretching vibration. This particular peak was more prominent in the CS-LA material than it was in CS. Also, the peak around 1650 cm^{-1} corresponding to a C=O stretching vibration was not observed in CS-LA. Furthermore, the presence of two peaks in the region 1550–1650 cm^{-1} was consistent with the presence of an ion pair between the amino and carboxyl groups. These characteristics were observed in two the different types of CS and CS-LA regardless of their molecular weight, which indicated that the amino groups of CS had formed an ion pair with the carboxyl groups of LA (**Fig. 2**). The modification ratios of LA to the amino groups of CS250 and CS600 were determined 56.6±3.5 and 68.7±3.5%, respectively by measuring the viscosity (**Table 1**).

3.2. Particle size of dispersion

Table 4 shows the particle size and relative width of particle size (R_W) of the CS-LA dispersion. When CS was used as a control to prepare dispersions, it was dissolved in acetic acid and the dispersion became solution. The CS250-LA dispersion showed a smaller mean particle size (20 ± 1 μm) than that of the CS600-LA dispersion (92 ± 15 μm), which was attributed to the lower molecular weight CS250 as well as lower modification ratio of

299 CS250-LA.

300

301 ***3.3. Characterization of the polymer films***

302 ***3.3.1 Surface morphology of the polymer films***

303 Consideration of the SEM images revealed that the surface of the CS600-LA film
304 was rough, whereas the surface of the CS250-LA films was smooth (**Fig. 3**). This difference
305 in the surface morphology of the two films was attributed to the smaller mean particle size of
306 the CS250-LA dispersion. The surface morphology of the polymer films appeared to be
307 independent of the concentration of glycerin, because changes the concentration of glycerin
308 had no discernible effect on the surface morphology of the polymer films. In contrast to the
309 CS-LA films, the CS films showed a smooth surface, which was attributed to the CS materials
310 being in solution.

311

312 ***3.3.2 Acid stability of polymer films***

313 **Figure 4** shows the results of the disintegration tests for the different films in 1st
314 fluid (pH 1.2). The CS250 films prepared using dispersions containing 0.5, 1.0 and 1.5%
315 glycerin disintegrated in 10.3, 16.0 and 17.7 h, respectively (**Fig. 4A, closed bar**). The CS600
316 films prepared using dispersions containing 0.5, 1.0 and 1.5% glycerin disintegrated in 9.0,
317 14.0 and 17.0 h, respectively (**Fig. 4B**, **closed bar**). The fact that the CS films prepared using
318 dispersions containing 1.5% glycerin could maintain their form for 17 h was attributed to the
319 thick nature of the films (about 100 μm). In contrast, CS250-LA films prepared using
320 dispersions containing 0.5, 1.0 and 1.5% glycerin showed longer disintegration times of 22.0,
321 39.3 and 42.3 h, respectively (**Fig. 4A, open bar**). CS600-LA films prepared using
322 dispersions containing 0.5, 1.0 and 1.5% glycerin also showed longer disintegration times of
323 47.0, 59.0 and 62.7 h, respectively (**Fig. 4B, open bar**). These results demonstrated that the

disintegration times of the CS-LA films were more than twice as long as those of the CS films. Based on these results, it was obvious that the derivatization of CS led to greater stability under gastric condition. In fact, even though the coating thickness of these formulations was only a few micrometers, these data suggested that formulations coated with an appropriately thick layer of CS-LA could pass through the stomach without releasing their drug. Also, an increase in the glycerin concentration led to an increase in the disintegration time in the acidic test fluid. This result could be attributed to an increase in the plasticization effect of glycerin, which enhances the flexibility of the film and provides it with greater acidic stability.

3.3.3 Degradation of the polymer films

Figure 5 shows the weight loss details of the polymer films in phosphate buffered solution (pH 6.0) both in the presence and absence of *β*-glucosidase after 24 h. Although all of the CS250 films were disintegrated to some extent in the absence of *β*-glucosidase (**Fig. 5A, closed bar**), they all completely disintegrated in the presence of *β*-glucosidase (**Fig. 5A, open bar**). The CS600 films were also disintegrated to some extent in the absence *β*-glucosidase (**Fig. 5B**). However, a significant weight loss was only observed for the CS600 films containing 0.5% glycerin in the presence of *β*-glucosidase (**Fig. 5B**). It has been reported that lower molecular weight CS is more easily degraded than higher molecular weight CS. [14] As for CS-LA films, all of the CS250-LA films showed high weight losses in the presence of *β*-glucosidase regardless of their glycerin concentration (i.e., 84.8, 90.5 and 84.9% with 0.5, 1.0 and 1.5% glycerin, respectively) (**Fig. 5C, open bar**), whereas the weight losses in the absence of *β*-glucosidase were much lower (i.e., 20.9, 39.0 and 49.4% with 0.5, 1.0 and 1.5% glycerin, respectively) (**Fig. 5C, closed bar**). In contrast, only the CS600-LA containing 0.5% glycerin showed a significant weight loss (**Fig.5D**). This result was attributed to the CS250-LA material being more easily recognized by the *β*-glucosidase than CS600-LA

because the modification ratio of the amino groups on CS250-LA was lower (56.6±3.5%) than that of CS600-LA (68.7±3.5%) (**Table 1**). However, given that CS can be broken down in a number of different ways in the colon by a number of different enzymes, it is envisaged that the CS-LA coating film would be degraded to a greater extent *in vivo*.

3.3.4 Mechanical properties of polymer films

Figure 6 shows the TS and EB results for the of the polymer films. The black dot lines in these graphs indicate the measured values for the Chitocoat film as a control. The CS and CS-LA films displayed very similar tendencies in terms of their TS and EB values. Although the EB values appeared to be independent of the glycerin concentration, the TS values decreased with increasing glycerin concentration, which indicated that the CS and CS-LA films could be made more flexible depending on their glycerin concentration. A similar tendency was also observed as the molecular weight of the CS increased, which indicated that the both films could be made more flexible depending on the molecular weight of the CS material. These results therefore showed quite clearly that CS-LA dispersions containing more than 1.0% (w/w) glycerin could be used to form a coating film with suitable flexibility properties, because the films showed mechanical properties (i.e., TS about 0.7 MPa; EB about 13%/mm^2) almost equal to those of Chitocoat films.

3.4 Dissolution test using disks

The results provided above for the evaluation of the polymer films suggested that CS250-LA dispersions containing more than 1.0% (w/w) glycerin could be used to make films with suitable acid stability, colonic degradability and flexible film properties. With this in mind, we proceeded to evaluate the actual ability of this CS250-LA dispersion containing 1.0% (w/w) glycerin to suppress the release of a drug from a disk coated in a film of

CS250-LA. **Figure 7** shows the results of a dissolution test using coating disks. In a test fluid at pH 1.2, disks coated in Chitocoat released drug more rapidly than the other disks. This result was attributed to the Chitocoat films not working effectively as coating films because they disintegrated under the acidic conditions. **Table 5** shows the drug permeation rates from the disks, which were calculated from the slopes of lines. In the test fluid at pH 1.2, the values for the Chitocoat and CS250-LA disks were 7148 and 195 mg/h/cm^2・μm, respectively. This 37-fold difference in the results effectively highlights the increase in the acid stability of CS resulting from the derivatization process. This result therefore shows quite clearly that the release of the drug in the stomach can be sufficiently suppressed using the CS250-LA coating dispersion. Furthermore, the drug permeation rates of the Chitocoat and CS250-LA disks in a test fluid at pH 6.8 were 390 and 195 mg/h/cm^2 μm, respectively. The value for the Chitocoat disks was therefore 2-fold larger than that of the CS250-LA disks. This difference was attributed to an increase in the hydrophobicity of the CS derivative following its modification with LA, which led to a reduction in the permeation of the drug to the buffer. These results suggest that CS250-LA coating films could better suppress the release of drug molecules from formulations in the small intestine than Chitocoat films, and that CS250-LA coating films could therefore be used to effectively deliver drugs to the colon.

4. Conclusions

CS250-LA dispersions containing more than 1.0% (w/w) glycerin gave coating films with acid stability, colonic degradability and flexible properties suitable for the delivery of drug molecules to the colon. The release of APAP from disks coated with CS250-LA dispersions containing 1.0% (w/w) glycerin was significantly suppressed in test fluids at pH values of 1.2 and 6.8, which suggested that drugs could be effectively delivered to the colon

by coating this dispersion onto formulations without requiring overcoating. Based on the results of this study, further *in vivo* work using IBD model mice should be needed to evaluate the usefulness of this dispersion for the coating of tablets or granules containing 5-aminosalicylic acid (5-ASA) to achieve colon- specific drug delivery in comparison to commercially available 5-ASA products.

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436

437 **Table 1. Physicochemical properties of CS-LA.**

	Molecular weight of CS (kDa)	Solubility of CS in 0.15% (w/v) acetic acid (mg/mL)	Modification ratio of CS-LA (%)
CS250-LA	190–310	8.68±0.07	56.6±3.5*
CS600-LA	600	9.32±0.10	68.7±3.5

438 Solubility of CS represents the mean ± S. D. (n=3). *, $p < 0.05$, compared with
439 CS600-LA.
440

441 **Table 2. Composition of the CS solution and CS-LA dispersions.**

Composition	Ratio (%, w/w)
CS or CS-LA	1.0
Glycerin	0.5, 1.0, 1.5
Tween 80	0.1
Acetic acid	0.15
Distilled water	Adjust to 100

442

443 **Table 3. Composition of Chitocoat.**

Composition	Ratio (%,w/w)
CS	2.0
Glycerol esters of fatty acids	1.0
Glycerin	1.0
Acetic acid	0.6
Distilled water	95.4

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446 **Table 4. Particle size and relative width of particle size (R_w) properties of each CS-LA**

447 **dispersion.**

	d_{10} (μm)	d_{50} (μm)	d_{90} (μm)	R_w
CS250-LA	6.1±3.3	20±1	53±12	2.3±0.8
CS600-LA	21±5	92±15	222±29	2.2±0.3

448 Each value represents the mean ± S.D. (n=3).

449

450 **Table 5. Permeation rates of APAP from the Chitocoat and CS250-LA coated disks.**

	Permeation rate (mg/h/cm^2・(μm))	
	pH1.2	pH6.8
Chitocoat	7148 ± 4465	390 ± 70
CS250-LA	195 ± 92*	195 ± 136

451 *, $p < 0.05$, compared with Chitocoat coated disks.

452

Figure captions

Figure 1. FT-IR spectra of CS, LA and CS-LA. Figures represent (A) CS250 and (B) CS600.

Figure 2. Chemical structure of CS-LA.

Figure 3. Scanning electron microscope images of the surfaces of the CS and CS-LA films.

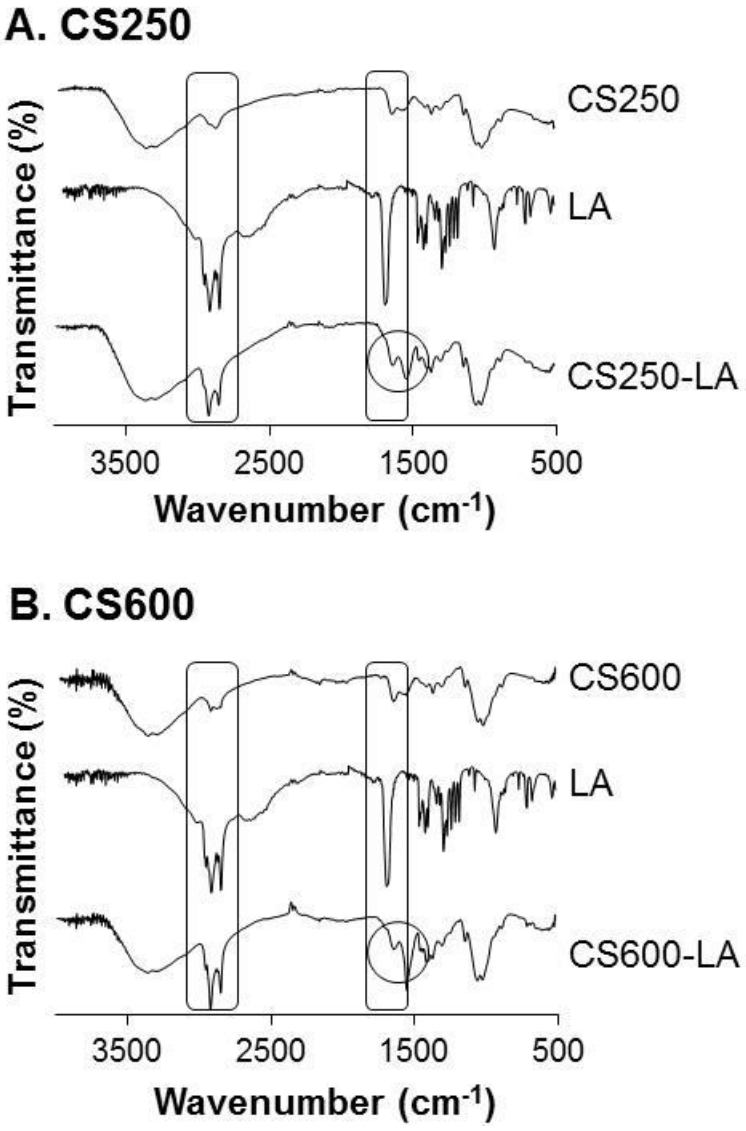
Figure 4. Disintegration times of the CS and CS-LA films in 1st fluid for the disintegration test (pH 1.2). (A) CS250 and (B) CS600. Each column represents the mean ± S.D. (n=3). *, $p < 0.05$ and **, $p < 0.01$, compared with each CS film.

Figure 5. Weight loss of the (A) CS250 film, (B) CS600 film, (C) CS250-LA film and CS600-LA film in pH 6.0 phosphate buffer both with and without 1.0 % (w/v) *β*-glucosidase after 24 h. Each column represents the mean ± S.D. (n=3). *, $p < 0.05$ and **, $p < 0.01$, compared with each film in the absence of β-glucosidase.

Figure 6. Tensile strength and Elongation at break values of the (A) CS film and (B) CS-LA film. Black dot lines demonstrate the measured values of Chitocoat. Each column represents the mean ± S.D. (n=3).

Figure 7. APAP release behavior from Chitocoat and CS250-LA dispersion coated disks in test fluids at pH 1.2 and pH 6.8. Each symbol represents the mean ± S.D. (n=3).

Fig. 1


A. CS250
Transmittance (%)
CS250
LA
CS250-LA
3500
2500
1500
500
Wavenumber (cm-1)
B. CS600
Transmittance (%)
CS600
LA
CS600-LA
3500
2500
1500
500
Wavenumber (cm-1)

478

Fig. 2

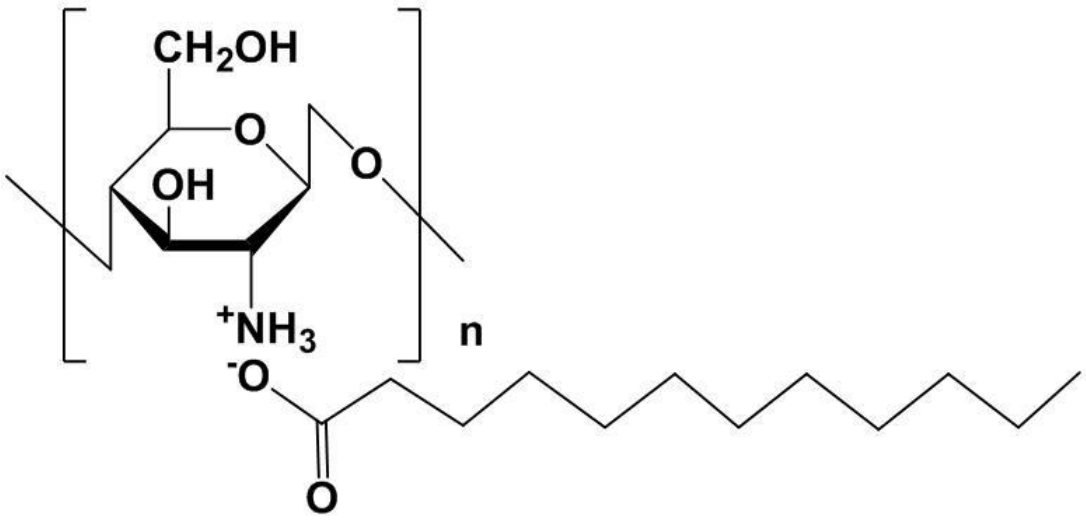


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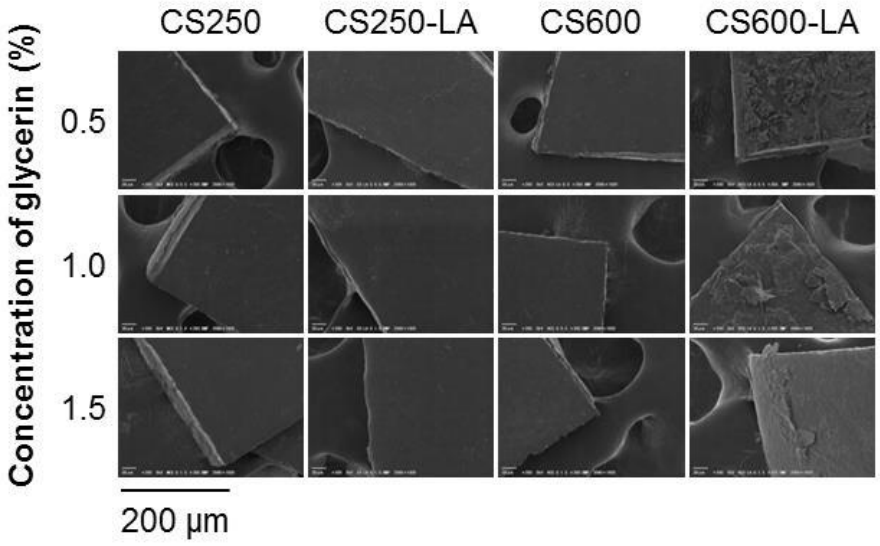
Fig. 3



480

Fig. 4

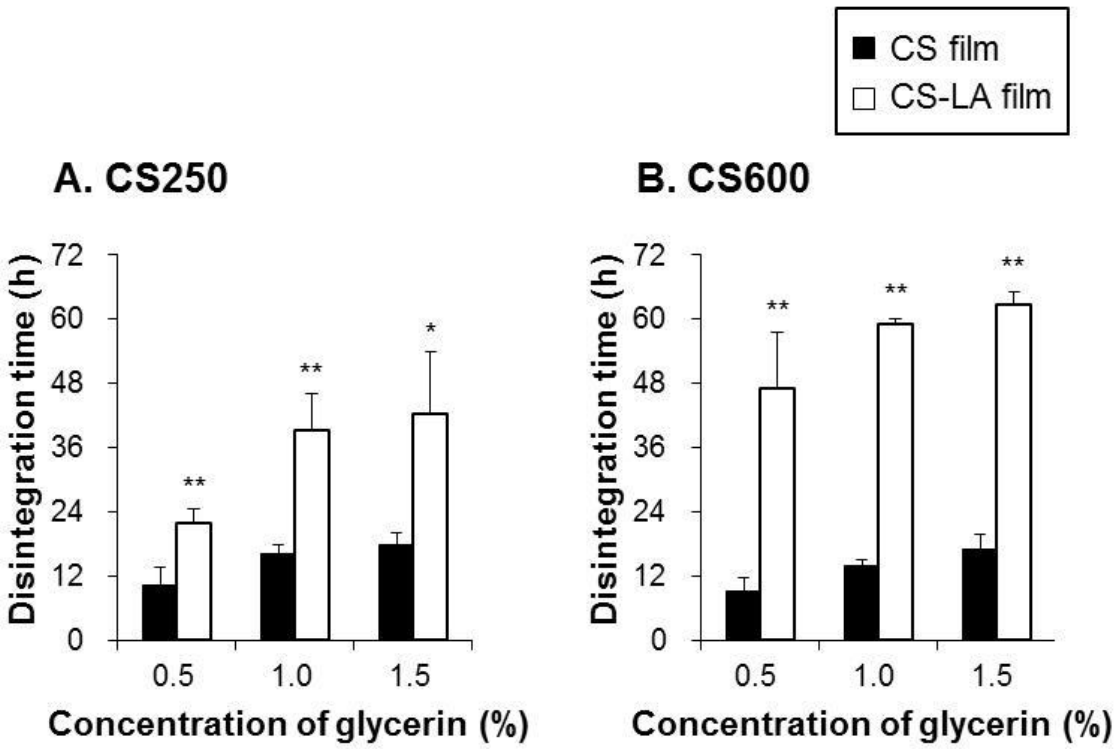


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Fig. 5

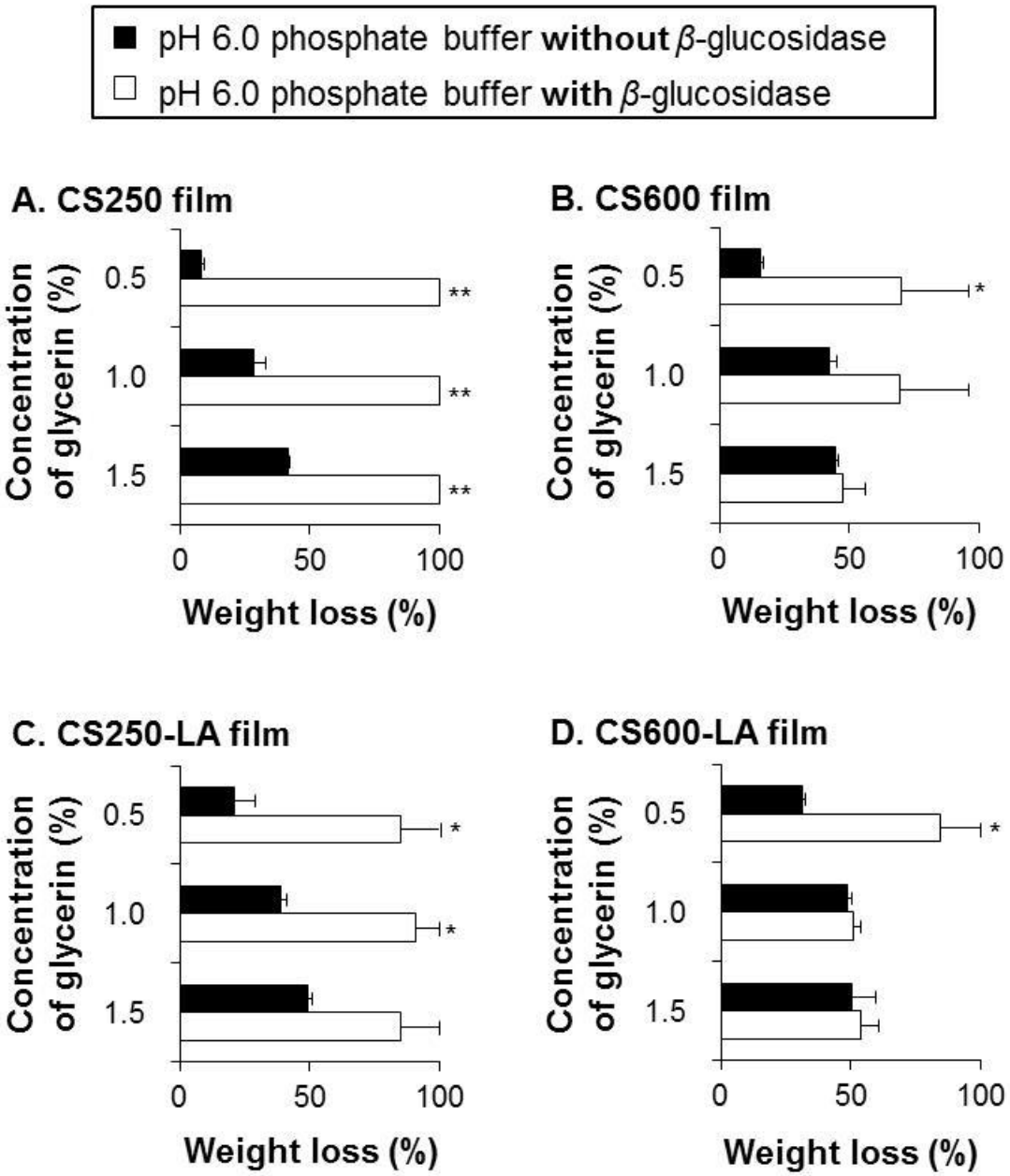

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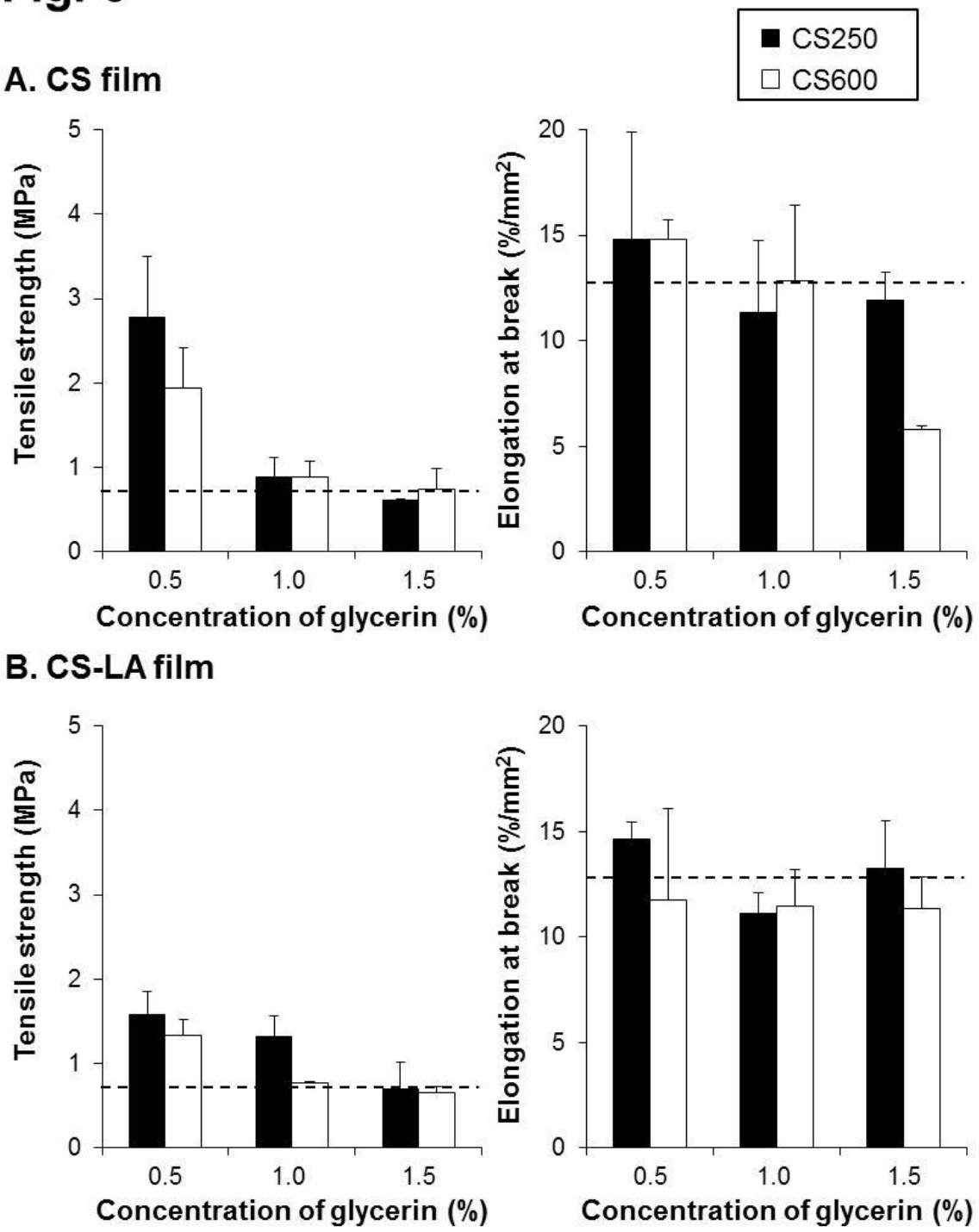
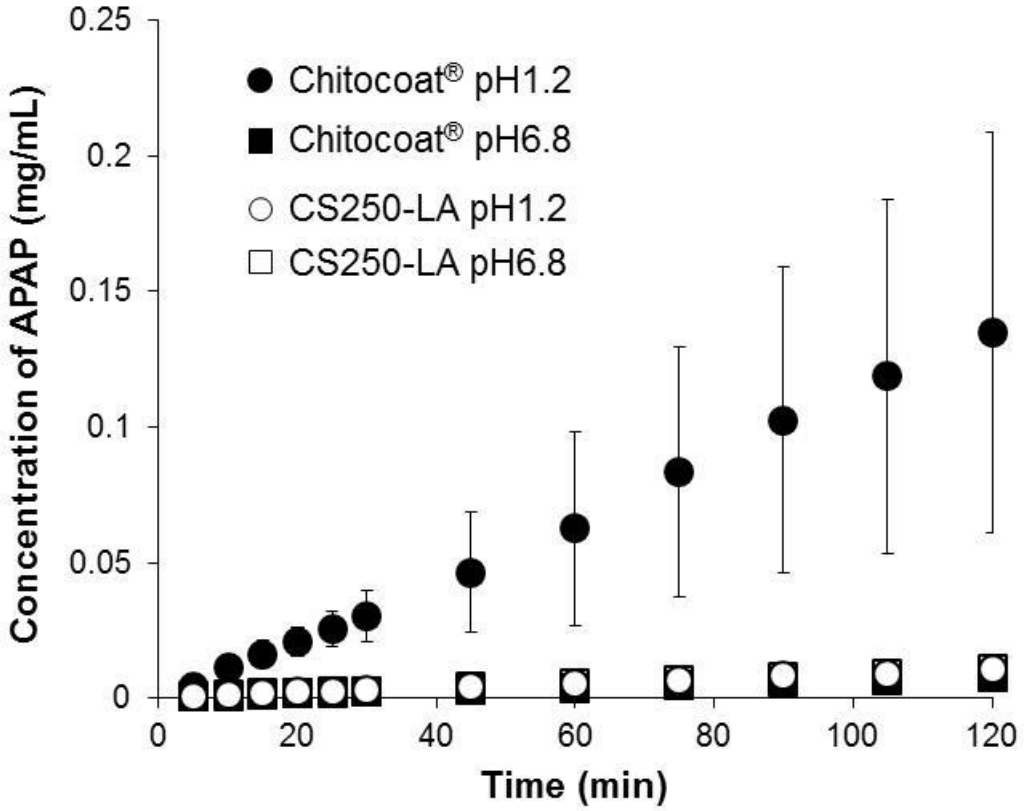
Fig. 6


A. CS film
CS250
CS600
Tensile strength (MPa)
Elongation at break (%/mm²)
Concentration of glycerin (%)
0.5
1.0
1.5
B. CS-LA film

491

492

Fig. 7


0.25
0.2
0.15
0.1
0.05
0
Concentration of APAP (mg/mL)
Chitocoat® pH1.2
Chitocoat® pH6.8
CS250-LA pH1.2
CS250-LA pH6.8
0
20
40
60
80
100
120
Time (min)

493