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# Disintegration Properties and Drug Release Profiles of Chondroitin Sulfate Films

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## Abstract

Film dosage form (FD) prepared using water-soluble polymers is an excellent technique by which drugs can be delivered to local disease sites. In this study, FD was prepared with the sodium salt of chondroitin sulfate (CHS), a natural polysaccharide, by the casting method. The disintegration profile of FD was assessed and the dissolution profile of the model drug from the dosage form was investigated. FDs containing atropine sulfate were prepared by using 4−6% of CHS. When the FDs were brought in contact with physiological saline, they swelled quickly and disintegrated resulting in CHS release into the medium. The model drug dissolved immediately from the FDs and almost the entire amount of the drug incorporated in the FD was dissolved after 5 min. FD prepared with CHS is an attractive dosage form that can be used for local drug delivery.

**Keywords**: Sodium Chondroitin Sulfate, Film Dosage Form, Film Disintegration, Atropine Sulfate, Drug Release Profile

## 1. Introduction

Chondroitin sulfate is widely present in mammalian connective tissues. It is a heterogeneous glycosaminoglycan formed of alternate and sulfated disaccharides of glucuronic acid and *N*-acetyl-D-galactosamine [1]. The sodium salt of chondroitin sulfate, CHS, has been used as an oral medicine or a supplement for the management of osteoarthritis [2-4]. By local application as an eye lotion, CHS is used in patients with dry eyes [5,6]. Recently, CHS is noted as a material for drug delivery system [7-9].

Film dosage form (FD) is a thin film containing active compounds. FD can quickly swell and disintegrate inside the bodily fluid when prepared using water-soluble polymers. Some polysaccharides form thin films after evaporation of the solvents in the solutions. Natural polysaccharide, such as sodium alginate or pectate, has been studied as base materials to prepare FD through which drugs can be efficiently delivered to the local disease sites [10-12]. Though the disintegration rate of the film matrix is an important factor which characterizes the dosage form, the degree of erosion is difficult to quantify. We previously reported, by a simple colorimetric assay using carbodiimide, the amount of polysaccharide containing uronic acids, such as alginic acid or pectic acid in aqueous solutions [13]. Furthermore, this assay was utilized to estimate the disintegration profiles in the test medium of FD prepared with the polysaccharide [14,15].

CHS is also a natural water-soluble polysaccharide; however, the characteristics of the film formed with CHS have been rarely studied. In this study, FDs were prepared with CHS solutions as the film base by the casting method. The disintegration profiles of FDs were then assessed by measuring the amount of CHS dissolved from each FD in the test medium. The dissolution profiles of the model drug from the FDs were investigated.

## 2. Materials and Methods

*2.1. Material.* As the film base, commercially available CHS (chondroitin 6-sulfate; Wako Pure Chemical Industries, Ltd., Osaka, Japan) was used. The other commercial product of chondroitin 6-sulfate (Nacalai Tesque Inc. Kyoto, Japan), CHS-N, was also used as a standard sample for the colorimetric assay. The film base solution was prepared with deionized water and the viscosity was measured with a viscometer (VM-1G-M, CBC Materials, Tokyo) at 20°C. Atropine sulfate (AP) was purchased from Tokyo Chemical Industry Co, Ltd. (Tokyo, Japan). Dexamethasone and hydroxylamine (HX) were purchased from Wako (Pure Chemical

Industries), Ltd. A water-soluble carbodiimide, 1-cyclohexyl-3-(2-morpholino ethyl) carbodiimide metho-p-toluenesulfonate (CMEC) was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). All other chemicals were of reagent grade and were obtained from commercial sources.

*2.2. FD Preparation.* FD was prepared as follows: 10 mL of 4−6% (w/w) CHS containing a model drug was dispersed in deionized water to prepare the film base solution. The mixture was thoroughly mixed by sonication and poured (3 g each) into individual plastic Petri dishes (diameter, 54 mm). The dishes were kept for 24 h at 37°C, after which the circular films formed were transferred into a desiccator. The thickness was measured at 10 points on each film using a micrometer (CLM1-15QM; Mitutoyo, Kawasaki, Japan) with a set pressure of 0.5 *N*. The measurements were taken using 3 films, and the mean thickness was calculated for each type.

*2.3. Film Disintegration Test.* A film was placed in a plastic dish, and 10 mL of physiological saline preheated to 37°C was added. The dish was then shaken (300 rpm) in an incubator (SI-300; As One Co., Osaka, Japan) set at 37°C. The medium (0.3 mL) was periodically removed using a plastic syringe and filtered through a syringe-driven filter unit (pore size, 0.45 μm). An equal volume (0.3 mL) of physiological saline at 37°C was added to the dish in the incubator to maintain a constant volume. Aliquots (0.1 mL) of the filtered solution were combined with 0.9 ml of ion-exchanged water in the test tubes before thoroughly mixing with a vortex mixer. The amount of CHS in each sample solution (1 mL) was measured using the method described below. Each test was performed in triplicate.

*2.4. CHS Assay in a Colorimeter.* The reagent solutions used were 20 mM HX in ion-exchanged water and 0.1 M CMEC in 2% pyridine-HCl buffer (pH 5.0). Aliquots (1 mL) of HX and CMEC reagents were added to 1 mL of the sample solution, followed by mixing with a vortex. Each mixture was incubated at 40°C for 20 min, after which 20 mM $FeCl_3$ in 0.1 M HCl (3 mL) was added. The absorbance of the solution in a quartz cell (1 cm light path) was measured at 480 nm using a spectrophotometer (UV-1200; Shimadzu, Kyoto, Japan). Each absorbance value was normalized to that of the blank reagent. For each test, a calibration curve was constructed using a fresh set of CHS standards.

*2.5. Drug Dissolution Test.* The sample solution was obtained by the same method described in the film disintegration test section. Next, 80-μL aliquots of the filtered sample solution were placed in micro test tubes (1.5 mL), to which 720 μL of methanol was added to precipitate the polysaccharide dissolved from the dosage form. Samples were mixed and

centrifuge (7,700 × *g*, 5 min; H-1300; Kokusan Co., Saitama, Japan). The supernatants were injected into the HPLC column. Each test was performed in triplicate.

*2.6. AP Assay.* The HPLC system (Hitachi Co., Tokyo, Japan) consisted of a pump (L-2130), UV-detector (L-2400), autosampler (L-2200), and chromate-integrator (D-2500) connected to a packed column (150 mm × 4.6 mm, Cosmosil 5C18-MS-II, Nacalai Tesque Inc.). To determine the concentration of AP, the assay was performed at ambient temperature using a mobile phase consisting of 6 mM phosphoric acid and acetonitrile (17:3) at a flow rate of 1.0 mL/min [16]. The detector wavelength was set at 215 nm.

## 3. Results and Discussion

To prepare FD by the casting method, an aqueous solution of CHS was poured onto a Petri-dish. The viscosity of 4−6% (w/w) CHS was low while pouring the base solution (Table 1). When 4% CHS was used as a film base, a 30-μm-thick film was obtained. FDs containing AP (1.5 mg/film) were prepared by using 4−6% CHS as shown in Figure 1. On the other hand, FDs containing dexamethasone (0.75 mg/film) were not obtained in all cases of 4−6% CHS. Therefore, the compounds incorporated in the FD were carefully selected because their addition to the base solution interfered with the film formation depending on the polysaccharide.

When FD was soaked in physiological saline at 37°C, it swelled quickly and then disintegrated resulting in CHS release into the medium. In the disintegration test, the amount of CHS dissolved was measured by the method which changed uronic acid within the polysaccharide to a hydroxamic acid derivative. As shown in Figure 2, the calibration curves for both CHS and CHS-N were in good agreement in the range of 0.1−1 mg. The coefficient of variation for the determination of 0.5 mg CHS was 3.3% (n=8).

Figure 3 shows the disintegration profiles of FDs containing AP. CHS was immediately dissolved in the test solution in association with the disintegration of FD. For the FD prepared with 4% CHS, 80% of the incorporated CHS dissolved by 1 min and the total amount of the film base was dissolved into the test medium at 3 min. Similar dissolution profiles were recognized with both 5% CHS and 6% CHS.

AP is a water-soluble compound; therefore, the drug dissolved immediately from the FD when it was brought in contact with the test medium. As shown in Figure 4, 1.2 ± 0.1 mg of

AP was dissolved at 1 min from the FD prepared with 4% CHS. The whole amount of AP incorporated in the FD was released after 3 min. The rapid drug dissolution rate from the FDs was also observed for the FDs prepared with 5% or 6% CHS and almost the entire amount of the drug incorporated in the FD was dissolved after 5 min. These results show that CHS quickly dissolves along with the complete disintegration of FD. Further, AP incorporated within the FD dissolves along with the disintegration of the film matrix after a restricted amount of FD is set in the aqueous media.

## 4. Conclusion

In this study, FDs containing model drug, AP, were prepared using CHS without dissolving them into organic solvents, heating them, controlling their pH, or adding other materials. When the FDs were brought in contact with physiological saline, the forms swelled quickly and then disintegrated resulting in the dissolution of both AP and CHS into the medium. FD prepared with CHS is an attractive dosage form that can be used for local drug delivery. It can also be a useful dosage form in patients due to its high solubility even in a restricted amount of the media.

## Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

**TABLE 1: Viscosity of CHS solution at 20°C.**

| Concentration (% w/w) | Viscosity (mPa・s) |
|---|---|
| 4 | < 10 |
| 5 | 12 |
| 6 | 15 |

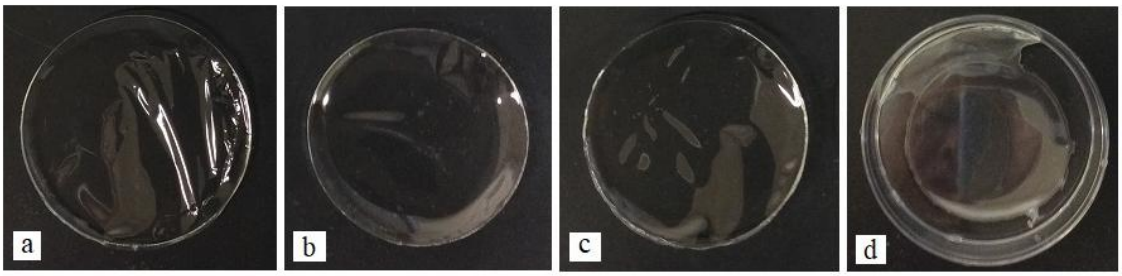


**FIGURE 1: Images of FDs prepared with the base solution containing the model drug.**

(a) 4% CHS containing AP, (b) 5% CHS containing AP, (c) 6% CHS containing AP,

(d) 6% CHS containing DM.

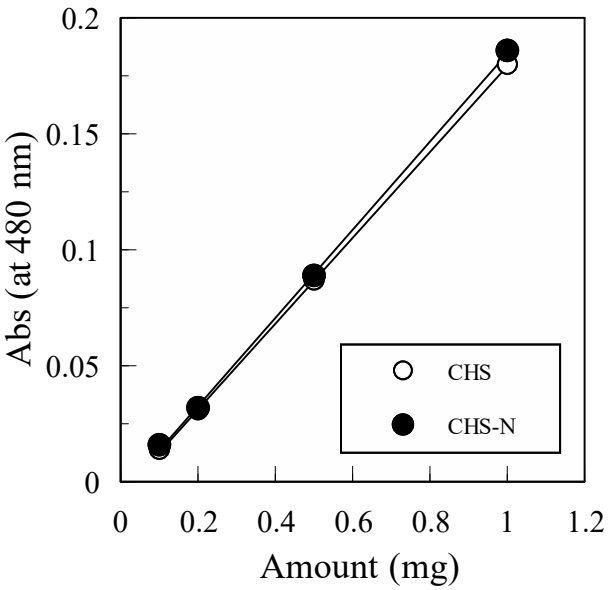


**FIGURE 2: Calibration curves of the CHSs.**

Calibration curves equation [Y: absorbance at 480 nm, X: amount of sample (mg), R: correlation coefficient].

CHS: $Y = 0.182\ X - 0.003$ ($R^2 = 0.9992$)

CHS-N: $Y = 0.188\ X - 0.003$ ($R^2 = 0.9991$)

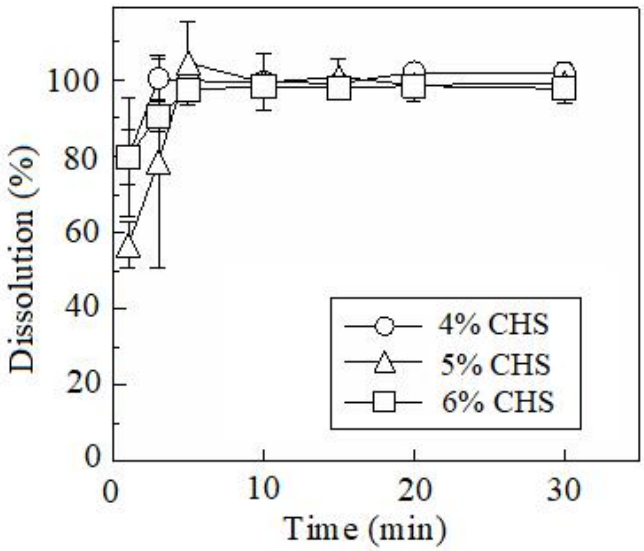


**FIGURE 3: Dissolution profiles of CHS from the FDs.**

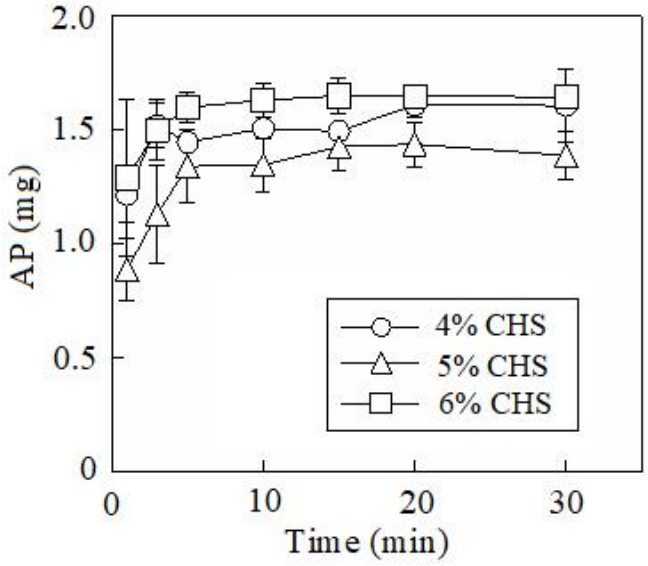


**FIGURE 4: Release profiles of AP from the FDs prepared with CHS.**

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