



Research Article

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Extraction of sennosides from solutions by chitosan and utilization of the extract for oral dosage form

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ABSTRACT

The extraction of sennoside (Sen) from a crude solution containing powdered senna leaf (SFP) by adsorption to chitosan (CS) was investigated. And the release profiles of Sen from the extract (CS-SFP) and from alginate-calcium gel beads (Alg-Ca) incorporating CS-SFP were examined. Sen adsorbed to CS was released into solution by electrostatic interactions. Negligible amounts of Sen were released into deionized water or ethanol, but significant amounts were released into physiological saline or phosphate buffer. Two species of Sen, SenA and SenB, were released from CS-SFP, and the amount released increased proportional to the amount of SFP used to prepare the CS-SFP. In dissolution tests, Alg-Ca incorporating Sen-SFP swelled without disintegrating, and SenA and SenB were simultaneously released from the Alg-Ca. And about 80% of the SenA contained in the Alg-Ca was released after 120 min. These results suggest that when CS-SFP is administered orally, Sen may be released immediately from the complex and if necessary, the release rate could be controlled by incorporating Sen into Alg-Ca and by modifying the gel matrix.

Key words: Sennoside, Chitosan, Extraction, Alginate gel bead, Constipation.

INTRODUCTION

Constipation is a symptom caused by the colonic diseases, mental stress, insufficient intake of dietary fibers, or as a side effect of drugs such as morphine. Several drugs improve the peristaltic movement of the intestines. For example, sennoside (Sen) has been used as a laxative, and is obtained from plants, such as *Sennae Folium* or *Rhei Rhizoma*, used for herbal remedies [1-3]. Sen is contained in commercial health teas prepared from the stem of senna [4,5], and powdered senna leaf (JP XV; *sennae folium pulveratum*, SFP) is used for the treatment of constipation. Several stereoisomers of Sen are present in Senna, and Sen A (SenA) and Sen B (SenB) are known to be the main active compounds exhibiting laxative properties. Both SenA and SenB change the rheinanthrones of intestinal bacteria, and then act as laxatives in the colon when orally administered [6,7].

The biocompatibility of chitosan (CS), a natural cationic polysaccharide, has been studied to determine its utility in formulations [8]. It is known that CS interacts with anionic compounds and contributes to their stability by electrostatic complex formation [9,10]. Recently, CS has been used as a carrier for drug delivery [11,12]. On the other hand, alginic acid is an anionic polysaccharide that forms a cured gel in the presence of divalent or trivalent cations. For example, calcium-induced alginate (Alg-Ca) gel beads can incorporate compounds such as drugs or enzymes in the gel matrix, and their utility for controlled drug release has been studied [13,14].

CS and alginic acid are safe and widely used as food additives. In addition, these polysaccharides are not absorbed from the gastrointestinal tract, so they are a source of dietary fiber [15]. CS is also used in alternative therapy: for example, as a candidate for prevention of hyperlipidemia, since oral administration of CS decreases serum cholesterol levels [16-18]. This effect was observed when CS salt, prepared using a weak acid, was administered orally to rats [19]. Therefore, a CS salt prepared from CS and an acidic drug should show pharmacodynamic action of the drug released

from the CS salt.

In the present study, we extracted Sen from an aqueous solution by adsorption to CS. The resulting powder was called CS-Sen. The extract (CS-SFP) was prepared by extracting Sen from a crude solution containing SFP, comparable to the preparation of CS-Sen. Oral administration of CS-Sen and its transfer to the gastrointestinal tract might result in release of Sen and a laxative effect. Therefore, the release profiles of Sen from CS-Sen and CS-SFP were investigated. In addition, we devised a Sen/Alg-Ca formulation compatible with oral intake, and studied the release of Sen.

EXPERIMENTAL SECTION

Materials

Two isomers of Sen, SenA and SenB, as well as chitin, alginic acid and curdlan were obtained from Wako Pure Chemical Co. (Osaka). SFP was obtained from Nakajima Shoyaku Co. (Kyoto). Pursennid, a tablet form containing Sen, was purchased from Novartis Pharma K.K. (Switzerland). CS (fine powder, degree of deacetylation: 75–85%) was obtained from Kimitsu Chemical Industries Co. Ltd. (Tokyo). All other chemicals were of reagent grade.

Adsorption test

Two milligrams of polysaccharide were added to 20 ml of SenA or SenB solution prepared in deionized water (10 µg/ml) and incubated with shaking at 37°C for 0.5 h (AS ONE, shaking incubator SI-300, Osaka). The suspension was centrifuged at 10,000 rpm for 5 min, then the supernatant was filtered (pore size: 0.45 µm) and injected onto an HPLC column [3]. The HPLC system consisted of a LC-6A pump (Shimadzu Co., Kyoto), a packed column (150 mm × 4.6 mm, Cosmosil 5C₁₈-MS-II; Co., Kyoto), and a SPD-6A UV detector (Shimadzu Co.). Quantification of Sen was conducted at ambient temperature using an eluent consisting of 0.1% phosphoric acid and acetonitrile (4:1) at a flow rate of 0.8 ml/min. The detector wavelength was 254 nm. The amount of Sen adsorbing to the polysaccharide was calculated from the amount of Sen added and the amount present in the residue. All tests were performed in triplicate.

Preparation of CS-Sen

CS (5 g) was added to 500 ml of Sen solution (10 µg/ml) and the mixture was stirred for 1 h at room temperature. The suspension was centrifuged at 3,000 rpm for 10 min. The precipitate was collected and washed three times with ethanol, dried in a dish, then desiccated under vacuum in the presence of P₂O₅.

Preparation of CS-SFP

Three preparations of CS-SFP containing different amounts of SFP were prepared as follows. Five hundred ml of deionized water containing dispersed SFP (2, 4 or 8 mg/ml) were incubated at 37°C for 1 h, then the supernatant liquid was filtered. CS (1.0 g) was added to 100 ml of each supernatant, stirred for 1 h, then the suspension was centrifuged at 3,000 rpm for 10 min. The collected precipitate was treated as above for the CS-Sen preparation; the obtained powder was called CS-SFP.

X-Ray diffractometry

Powder X-ray diffractometry was conducted with an automatic diffractometer (MXP3; MAC Science Ltd., Yokohama) using a voltage of 40 kV and a current of 20 mA. The scan rate was 2°/min over a 2 theta range of 5–100°. The X-ray diffraction data were interpreted using a computer program (Bruker AXS K.K., Yokohama).

Release of Sen from CS-Sen and CS-SFP

Physiological saline, deionized water, 5 mM phosphate buffer (pH 7.0) and ethanol were used as the test solutions. One hundred milligrams of CS-Sen or CS-SFP were added to 10 ml of the test solution and incubated with shaking at 37°C. After 30 min and again after 2 h, 0.2 ml of each test sample was removed and the amount of Sen released was measured using HPLC. All tests were performed in triplicate.

Preparation of Alg-Ca containing CS-SFP

Alg-Ca containing CS-SFP was prepared as follows: 0.4 g of CS-SFP was dispersed in 9.6 g of 1.5(w/w)% sodium alginate solution with agitation. An aliquot of this solution (2 g) was dropped into 10 mL of 0.1 mol/l CaCl₂ and left to stand at room temperature for 1 h. The beads were washed twice with 50 ml distilled water, dried at 37°C for 24 h in a culture dish, and then desiccated under vacuum in the presence of P₂O₅.

Release profile of Sen from Alg-Ca containing CS-SFP

Gel beads containing CS-SFP (0.8 g) corresponding to 2 g of hydrogel were added to 20 ml of physiological saline and incubated with shaking at 37°C. A 0.2 ml aliquot of the solution was removed periodically and the amount of Sen released was determined using HPLC as described above. All tests were performed in triplicate. In addition, the drug release profile from a commercial tablet preparation containing Sen, Pursennid, was determined using a JP XIV

dissolution test apparatus (paddle method, 150 rpm, 37°C, 500 ml physiological saline).

RESULTS AND DISCUSSION

Dispersal of CS powder into Sen solution caused the CS to gradually change from pale yellow to brown, and the concentration of Sen in the solution decreased. In the case of an acidic polysaccharide, alginic acid or a neutral one, curdlan, this color change was not observed. The adsorption of SenA or SenB to several polysaccharides is shown in Figure 1. About 50% of SenA or SenB adsorbed to CS dispersed in the solution. In the case of partially deacetylated chitin, about 40% of each Sen was adsorbed, whereas no adsorption occurred to alginic acid or curdlan. These results suggest that Sen adsorbs to CS by electrostatic interactions. The X-ray diffraction patterns obtained from SenA, SenB, two species of CS-Sen, and CS-SFP are shown in Figure 2. SenA and SenB exhibited a characteristic diffraction pattern, whereas CS-SenA, CS-SenB and CS-SFP provided patterns lacking the SenA or SenB diffraction peaks. This result shows that the crystal form of Sen is a minor component of CS-Sen.

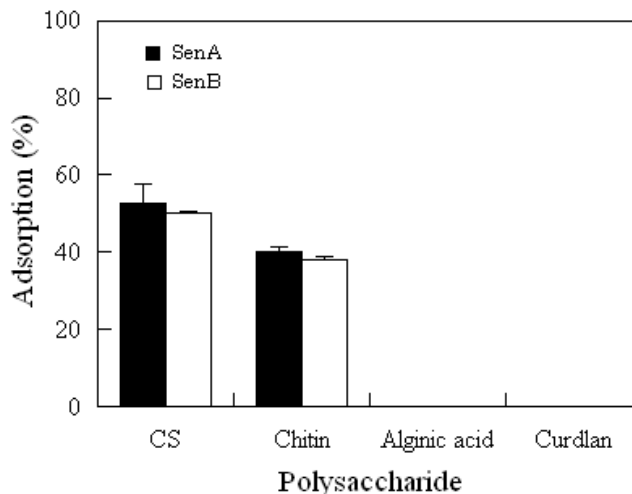


Fig. 1 Adsorption of Sen to polysaccharides

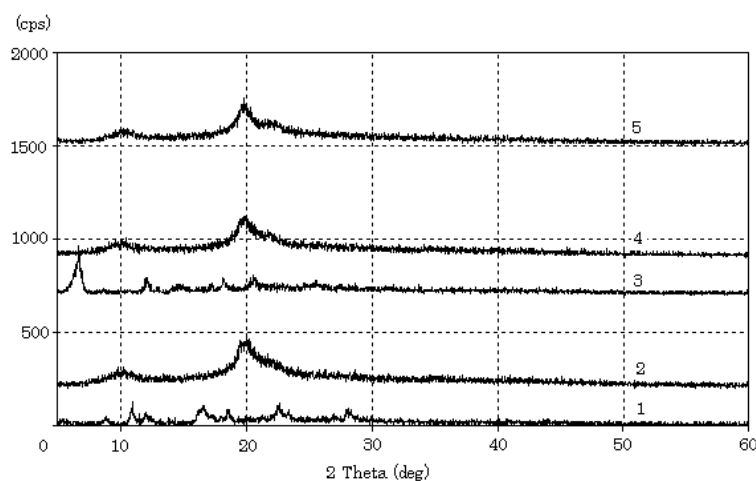


Fig. 2 X-ray diffractograms of Sen and CS-Sen complexes
1: SenA, 2: CS-SenA, 3: SenB, 4: CS-SenB, 5: CS-SFP

The amount of Sen released from CS-Sen powder in the various solutions after 30 min incubation is shown in Figure 3. Dispersion of CS-SenA in deionized water or ethanol resulted in little desorption of SenA, whereas significant amounts desorbed when CS-SenA was dispersed in either physiological saline or phosphate buffer (pH 7.0). The amount released after 30 min or 2 h was the same. Comparable results were obtained with CS-SenB. These results show that CS-Sen rapidly releases Sen due to ion-exchange reactions.

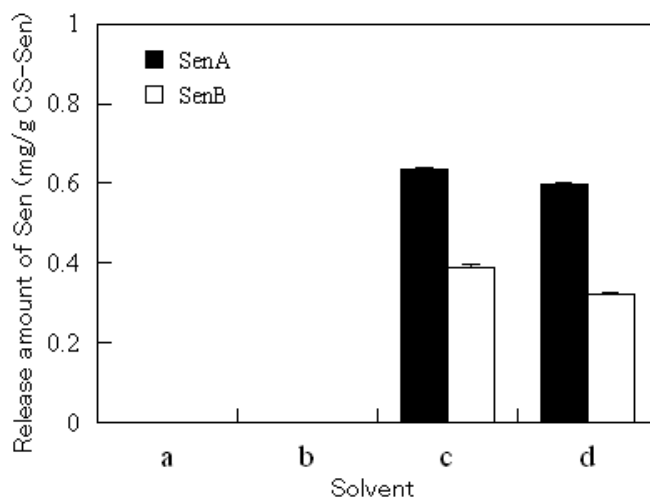


Fig. 3 Amounts of SenA and SenB released from CS-Sen complex
a: deionized water, b: ethanol, c: physiological saline, d: phosphate buffer (pH 7.0)

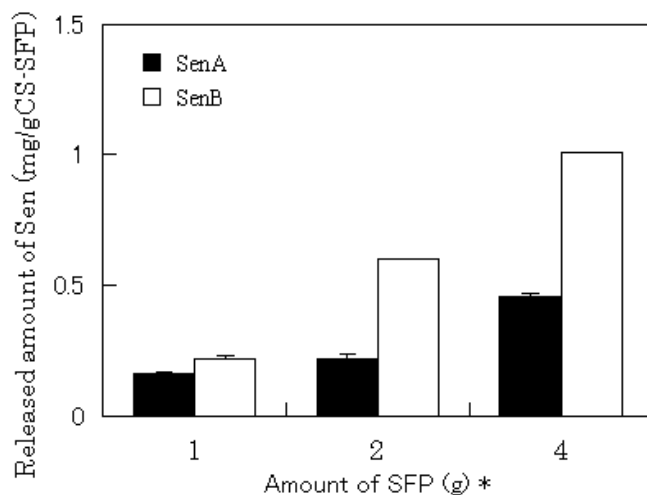


Fig. 4 Amounts of Sen released from CS-SFP
 *) Amount of SFP added to the solution (500 ml) for the preparation of CS-SFP

When SFP is dispersed in deionized water, various components of SFP dissolve into the medium. In the present study, CS powder was added to the crude solution, therefore, CS-SFP would contain components such as SenA, SenB, and others able to adsorb to CS. The amounts of Sen A and Sen B released from CS-SFP in physiological saline are shown in Figure 4. Both SenA and SenB were released from CS-SFP, and the amount released increased according to the amount of SFP used to prepare CS-SFP. In all cases, the amount of SenB released from Sen-SFP was larger than the amount of SenA. This result might reflect the amount and composition of Sen in the crude solution, although the ratio of SenA to SenB in Sen could not be determined directly by HPLC without purification due to the presence of many other SFP components in the solution.

Herbal medicines such as SFP, or tablets containing SenA and SenB, are taken orally to treat constipation. For example, about 80% of the Sen contained in Pursennid is released within 1 h upon disintegration of the tablet after a short lag time (10 min). In the present study, Alg-Ca incorporating Sen-SFP was prepared as an oral formulation. The release profiles of Sen from Alg-Ca are shown in Figure 5. In the dissolution test, Alg-Ca swelled without disintegration, and both SenA and SenB were simultaneously released. In the case of SenA, about 80% of the SenA contained in Alg-Ca was released by 120 min. On the other hand, the release of SenB was faster than that of SenA, with over 90% of the SenB contained in Alg-Ca released by 90 min. Each Sen dissociates from CS-SFP and diffuses in the alginate gel matrix, which the dissolution medium permeates. The difference in the rate of release between SenA and SenB may be attributed to the solubility of each Sen.

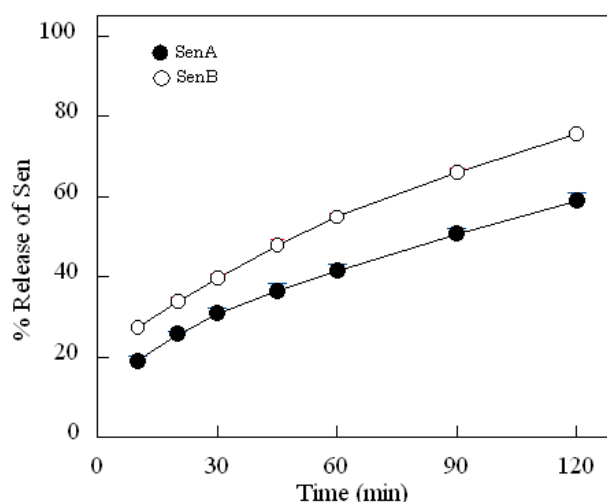


Fig. 5 Release profiles of Sen from Alg-Ca containing CS-SFP in physiological saline

Sen and herbal medicines such as SFP are popular drugs for the treatment of constipation, and are valuable since they are strong laxatives. SenA and SenB are acidic drugs, and each forms a CS-Sen complex by adsorption to CS. CS-SFP is a powdered form prepared using CS and a crude solution containing SenA, SenB, and other SFP dissolved compounds. When CS-SFP is administered orally, Sen may be released immediately from the complex in the gastrointestinal tract. If necessary, the release rate could be controlled by incorporating Sen into Alg-Ca and modifying the gel matrix [20]. Therefore, CS-SFP or Alg-Ca containing Sen is anticipated to show promise as a medicine for the treatment of constipation.

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