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Drug Release from Xyloglucan Beads Coated with Eudragit for Oral Drug Delivery

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Xyloglucan (XG), which exhibits thermal sol to gel transition, non-toxicity, and low gelation concentration, is of interest in the development of sustained release carriers for drug delivery. Drug-loaded XG beads were prepared by extruding dropwise a dispersion of indomethacin in aqueous XG solution (2 wt.-%) through a syringe into corn oil. Enteric coating of XG bead was performed using Eudragit L 100 to improve the stability of XG bead in gastrointestinal (GI) track and to achieve gastroresistant drug release. Release behavior of indomethacin from XG beads *in vitro* was investigated as a function of loading content of drug, pH of release medium, and concentration of coating agent. Adhesive force of XG was also measured using the tensile test. Uniform-sized spherical beads with particle diameters ranging from 692 ± 30 to 819 ± 50 μm were obtained. The effect of drug content on the release of indomethacin from XG beads depended on the medium pH. Release of indomethacin from XG beads was retarded by coating with Eudragit and increased rapidly with the change in medium pH from 1.2 to 7.4. Adhesive force of XG was stronger than that of Carbopol 943 P, a well-known commercial mucoadhesive polymer, in wet state. Results indicate the enteric-coated XG beads may be suitable as a carrier for oral drug delivery of irritant drug in the stomach.

Key words: Xyloglucan, Bead, Oral drug delivery, Eudragit, Mucoadhesive

INTRODUCTION

The basic goal of drug therapy is to achieve steady-state blood or tissue concentration for an extended period of time that is both therapeutically effective and nontoxic. At present, formulation of drugs in sustained release dosage form is used to achieve this goal (Hosny *et al.*, 1998).

Materials that exhibit thermally reversible sol-gel transition are of interest in the development of sustained release vehicles with *in situ* gelation properties (Kawasaki *et al.*, 1999). Typically, poloxamer, a triblock copolymer composed of poly(ethylene oxide) and poly(propylene oxide), transforms into gel upon warming up to body temperature by undergoing sol-gel transition (Schmolka, 1972). Gelation of the polymer solution containing drug renders slow-release characteristics to the drug delivery system (Morikawa *et al.*, 1987). However, the application of poloxamer as a carrier for drug delivery is restricted due to the high concentration (20-30 wt.-%) required for gelation (Schmolka, 1972) and possible toxicity at some sites. These problems may be avoided through the use of natural polymers. Hydrogels of natural polymers, particularly polysaccharides, have been widely used for their unique advantages such as nontoxic, biocompatible, biodegradable, and abundant properties (Pitt, 1990).

A good candidate is the polysaccharide xyloglucan (XG), which also exhibits sol to gel transition in the required temperature region, and has additional advantages of recognized non-toxicity and much lower gelation concentration than that of poloxamer (Yuguchi *et al.*, 1997). The XG derived from tamarind seeds is composed of a (1-4)-β-D-glucan backbone chain, which has (1-6)-α-D-xylose branches that are partially substituted by (1-2)-β-D-galactoxylose. When XG is partially degraded by β-galactoxylose, the resultant product exhibits a thermally

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reversible transition from sol to gel between 22~27°C over the concentration range of 1~2 wt.-% (Shirakawa *et al.*, 1998; Miyazaki *et al.*, 1998). Previous studies for the pharmaceutical application of XG concentrated on the sustained drug deliveries using *in situ* gelling properties following the oral (Kawasaki *et al.*, 1999; Miyazaki *et al.*, 2001, 2003), rectal (Miyazaki *et al.*, 1998), intraperitoneal (Suisha *et al.*, 1998), ophthalmic (Burgalassi, 2000), percutaneous (Takahashi *et al.*, 2002) administrations of chilled XG solution. In order to utilize the XG for the oral drug delivery, the development of other dosage forms, which is more convenient in administration, is demanded. Bead-type of XG gel has several advantages such as the facility of drug loading, reduction of the risk of dose dumping, and facility of dose adjustment (Bodmeier, 1997; Bulgarelli *et al.*, 2000) besides the convenient administration. However, the stability of XG beads is lowered due to the solation of gelled spheres in the dilute condition of GI track. The special coating process will improve the stability of XG beads and also allow the colon-specific release of gastric mucosal irritant drug.

Specific Eudragit acrylic polymers have been developed for oral dosage forms with step-wise release of active ingredient in the digestive tract (Breitkreutz, 2000; Cole *et al.*, 2002; Fan *et al.*, 2001). Eudragit L 100 is insoluble below pH 5 and thus resistant to gastric fluid. By salt formation in the neutral to weakly alkaline medium of the intestinal fluid, the polymer dissolves step-wise at pH values above 6. These properties are what render Eudragit L 100 as the most popular material for enteric-coating and stabilizing agent for XG beads.

Meanwhile, studies related with XG such as hydrogels for ocular delivery of pilocarpine (Burgalassi *et al.*, 1996a) and mucoadhesive buccal patches (Burgalassi *et al.*, 1996b) showed it to have mucoadhesive properties comparable to those of polyacrylic acid. Recently, particular attention has been paid to mucoadhesive micro/nanoparticles that adhere to intestine mucus and therefore prolong their migration time and extend drug release (Chen *et al.*, 1996; Kawashima *et al.*, 2000; Lim *et al.*, 2000).

In this study, XG beads were prepared by thermosensitive gelation of XG aqueous solution and coated with Eudragit L 100. *In vitro* drug release behavior of the Eudragit-coated XG beads was investigated to assess their potential as a carrier for oral drug delivery. The proposed system is expected to provide several advantages: Firstly, gelation of the dilute aqueous solution of XG renders oral sustained drug delivery. Secondly, Eudragit-coating prevents the solation of XG beads and irritant drug leakage in the stomach, leading to intestinal drug release. Finally, the mucoadhesive property of XG enhances adhesion on the mucosal surface with dissolution of the Eudragit-shell of XG beads in the intestine, resulting in the delivery of a drug across the mucous membrane for an extended period of time. Therefore, the aims of this study are to improve the stability of XG bead in gastrointestinal (GI) track and to achieve gastroresistant sustained drug release.

MATERIALS AND METHODS

Materials

Xyloglucan (0107S; galactose removal content, 44%) was provided by Danippon Pharmaceutical Co.(Osaka, Japan). Indomethacin was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Eudragit L 100 and Carbopol 934 P were provided by Röhm Pharmaceutical Co. and Noveon (Cleveland, OH, U.S.A.), respectively. All other chemicals used were of reagent grade and used without further purification.

Preparation of indomethacin-loaded xyloglucan bead

A weighed amount of XG (1 g) was dispersed in 50 mL of distilled water for 30 min. The mixture was slowly homogenized by a mechanical stirrer (Techno Lab-system PL-SS20D) for 24 h at 200 rpm and 4°C. An appropriate amount of indomethacin was then dispersed into the resulting solution at various concentrations (0.5, 1.0, and 2.0 wt.-%), and then extruded drop-wise through a disposable syringe into corn oil, which was placed on a magnetic stirrer (200 rpm) and preheated to 40°C. Stirring rate and temperature were maintained constant throughout the curing time (30 min). After gelation, the beads were filtered and washed quickly with acetone to remove the corn oil. Subsequently, the beads were washed stepwise with 75/25, 50/50, 25/75, and 0/100% water/ethanol mixtures (V/V) to preserve the spherical shape, and air dried for 24 h.

Preparation of enteric-coated XG beads

The XG beads prepared above were transferred into ethanol solutions of Eudragit L 100 at various concentration of 2.5, 5.0, and 10 wt.-%, and coated for 30 min with stirring (500 rpm). The resulting coated-XG beads were filtered and air dried. This coating process was repeated five times.

Evaluation of drug content of XG beads

Amounts of indomethacin loaded in the XG beads were determined using UV-Vis spectrophotomer (Optizen 2120 UV). Preweighed beads were ground using a homogenizer (Ultra-Turrax™ Homogenizer T25, IKA) and incubated in ethanol solution. The amount of indomethacin entrapped was then determined by measuring the UV absorbance at λ=319 nm. Drug content (DC) was calculated based on

the weight of initial drug-loaded beads and the amount of drug entrapped in beads as follows (Shin *et al.*, 1998). All data are the mean of three determinations for each of the XG and Eudragit L 100-coated XG beads

$$DC(\%)=\frac{\text{weight of indomethacin in beads}}{\text{weight of initial indomethacin-loaded beadss}}\times 100$$

Particle size analysis

The particle size distribution was carried out by counting a sample of 50 beads per batch and measuring the diameter using a micrometer (Digimatic Micrometer 293, Japan).

Observation of scanning electron microscopy (SEM)

Surfaces of the XG and Eudragit L 100-coated XG beads were observed using a scanning electron microscope (JSM-5410LV, JEOL, Japan). The beads were mounted onto an aluminum stub and coated with gold/palladium using a JEOL JFC-110E Ion Sputter.

In vitro drug release studies

Appropriate amounts of indomethacin-loaded beads (about 10 mg) were precisely weighed, enclosed in teabags, and placed into 10 mL of buffer solution release media. Hydrochloric acid, phosphate, and acetic acid buffer solutions were used for simulated gastric condition at pH 1.2, simulated intestine condition at pH 7.4, and at pH 4, respectively. The media were shaken at 120 strokes/min using a shaking incubator at 37°C (SI-600R, JEIO Tech). At predetermined time intervals, 1 mL of samples were withdrawn from the release media, and then the remainder media were removed and replaced by fresh buffer solutions. The amount of indomethacin released from the beads was determined spectrophotometrically at 319 nm (UV-Vis spectrophotomer, Optizen 2120 UV). The *in vitro* release studies were performed in triplicate for each of the XG and Eudragit L 100-coated XG beads.

Measurement of adhesive force

XG film was used to measure the adhesive force with Carbopol 943 P film as a control. Adhesive force of XG was measured using the tensile tester (Rheometric Scientific Inc, U.K.) with a plastic (polystylene) plate. XG films and plastic plates were cut into 1 cm × 1 cm and 1 cm × 2.5 cm pieces, respectively. The film piece was placed on the surface of a plastic plate, prewetted with 30 μL water, and covered with another plastic plate. They were pressed under 500 g scale weight for 5 min before measurement. The peak force required to detach the film from the plastic plate was measured (Ahn *et al.*, 2001).

RESULTS AND DISCUSSION

Preparation of indomethacin-loaded XG beads

XG beads were prepared through thermally reversible sol-gel transition in response to small changes in the external temperature. Aqueous XG solutions containing different concentrations (0.5, 1.0, and 2 wt.-%) of indomethacin were dropped into corn oil preheated to 40°C, which resulted in the instantaneous formation of gelled spheres by thermo-sensitive gelation. Furthermore, in an attempt to improve the stability of XG beads and to obtain gastroresistant and intestinal drug release, the beads were coated with different concentrations of Eudragit L 100/ethanol solution (2.5, 5.0, and 10.0 wt.-%). Table I and II summarize the particle sizes and drug content of uncoated and coated XG beads, respectively, where the average diameter and drug content of beads were tabulated as a function of the weight ratio of XG to indomethacin. The designation "XGID series" was used for drug-loaded samples by varying feed ratios of XG to indomethacin, which correspond to 0.5, 1.0, and 2.0 wt.-% of indomethacin. Uniform-sized beads were obtained with particle diameters ranging from 692 ± 29 to 819 ± 50 μm depending on the indomethacin content. The particle size analysis data revealed that the mean particle diameter of the uncoated beads increased with the weight ratio of XG to indomethacin, and that the coated beads showed larger particle diameters than the uncoated ones. The amount of indomethacin introduced into the XG beads was determined at different weight ratios of polymer to drug. The drug-loading content increased with the ratio of polymer to drug. On the other hand, the drug-loading content of the coated beads was lower than that of the

Table I. Size and indomethacin loading content of xyloglucan bead

Sample	Feed weight ratio		Mean particle diameter (mm)	Drug loading content (DC) (%)
	xyloglucan	indomethacin		
XGID 25	1.0	0.25	692±29	3.9 ±0.3
XGID 50	1.0	0.50	739±43	14.73±0.8
XGID 100	1.0	1.00	819±50	27.77±1.1

The data prepresent Mean±S.D. (n=3).

Table II. Size and indomethacin loading content of Eudragit L 100-coated xyloglucan bead

Sample	Coating concentration (wt.-%)	Mean particle diameter (μm)	Drug loading content (DC) (%)
XGID 50	2.5	807±58	13.2±0.5
	5.0	807±79	13.9±0.6
	10	822±66	12.5±0.4

The data prepresent Mean±S.D. (n=3).

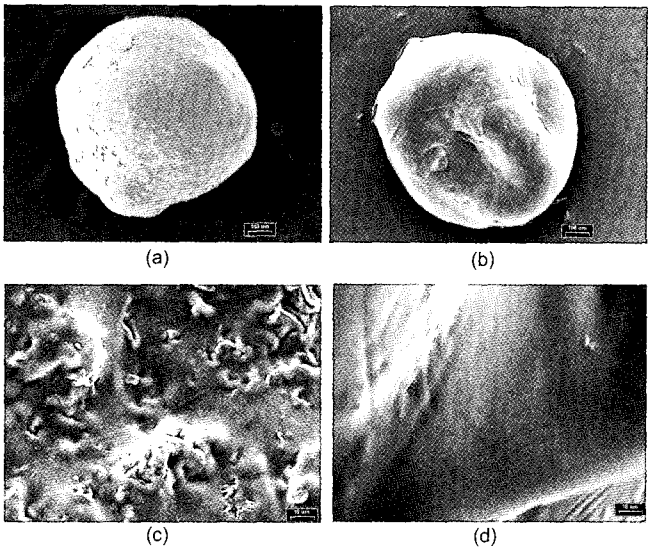

Fig. 1. Scanning electron micrographs of xyloglucan beads. (a) uncoated bead, (b) 5.0 wt.-% Eudragit-coated bead, (c) surface of the uncoated bead, (d) surface of the 5.0 wt.-% Eudragit-coated bead.

uncoated ones due to the washing with ethanol during the coating process.

Morphology of XG bead

SEM photographs of the XG bead (a and c) and Eudragit L 100-coated XG bead (b and d) are shown in Fig. 1. The XG beads were spherical-shaped, even if the spherical shape of bead in wet state was deformed slightly during drying. In contrast with very irregular surfaces of the uncoated XG beads, the Eudragit-coated XG beads had smooth surfaces due to the formation of thin layer of Eudragit on the bead shells [Fig. 1(c) and 1(d)].

In vitro release

Fig. 2a shows the release profiles of indomethacin from XG beads prepared through various polymer/drug weight ratios in pH 7.4 buffer solution. These data indicate that the release increases with loading content of drug in the beads, an indication that the level of drug release depends on the amount of drug entrapped in the beads. The XGID 100 beads containing 2.0 wt.-% drug show the highest drug release, with 50 % of the drug released within 50 min. On the other hand, while XGID 25 beads containing 0.5 wt.-% drug released same amount of the drug within 130 min. This difference may be due to the decrease in the polymer/drug ratio with an increase in the amount of drug loaded in the polymer, suggesting that higher amount of drug is released per unit area of exposed surface of the polymer matrix. The XGID 100 beads containing 2.0 wt.-% drug gave the lowest drug release in pH 1.2 buffer solution (Fig. 2b), indicating that drug release is restricted when the content of drug, with hydrophobic

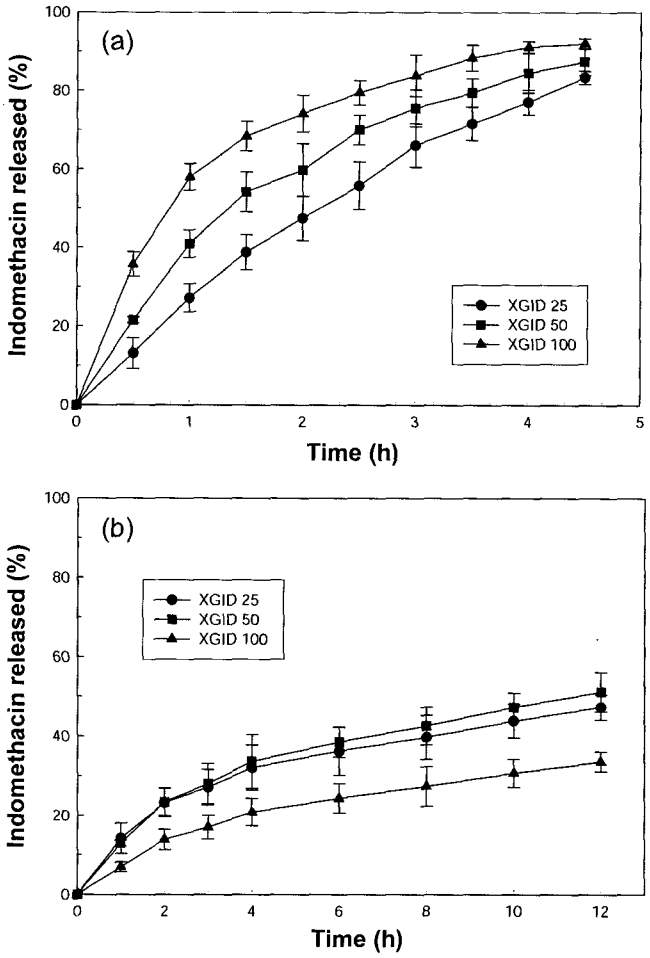

Fig. 2. *In vitro* release of indomethacin from xyloglucan beads as a function of drug content at 37°C (a) in pH 7.4 medium and (b) in pH 1.2 medium. Each point represents Mean±S.D. (n=3).

property, entrapped inside the beads is too high. *In vitro* drug release from XG gels has been reported to occur by diffusion through the aqueous channels of gel matrix (Miyazaki *et al.*, 1998). Under highly acidic condition, indomethacin is thought to have a greater tendency to associate with the polysaccharide chains rather than reside in the water channels of the gel due to the low solubility of its non-ionized form.

Fig. 3 shows the release profiles of indomethacin from Eudragit-coated XG beads as a function of pH at 37°C. As expected, the release rate increased with a midium pH, because the degree of ionization of carboxylic acid groups in the Eudragit L 100 increased with pH. However, in contrast to the rapid release of indomethacin from coated XG beads at pH 7.4, release of the drug at pH 1.2 and 4.0 was slow and showed sustained release characteristics. Eudragit L 100 is an anionic polymer based on methacrylic acid and methacrylic acid ester, which dissolves step-wise at pH values above 6.0. The dissolution of Eudragit L 100 on the core bead exhibits a remarkable contrast with this

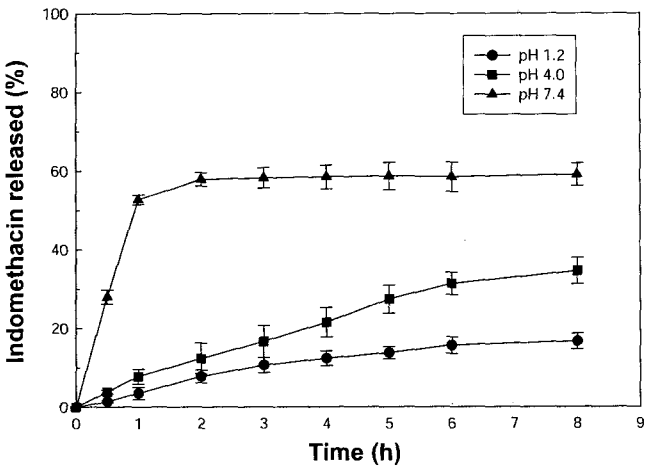

Fig. 3. *In vitro* release of indomethacin from 10 wt.-% Eudragit L 100-coated xyloglucan bead (XGID 50) as a function of pH at 37°C. Each point represents Mean±S.D. (n=3).

pH value as a datum point. At pH 7.4, Eudragit L 100 is fully dissolved and rapidly releases the drug from the core beads, whereas, at pH 1.2 and 4.0, the one is almost intact and releases the drug very slowly.

Fig. 4 shows the effects of Eudragit coating on the release of indomethacin from XG beads in pH 1.2 buffer solution at 37°C. The results show that indomethacin release from the Eudragit-coated XG beads was markedly retarded in comparison with that released from the uncoated XG beads, and that indomethacin release decreased as the concentration of the coating agent increased from 2.5 to 10.0 wt.-%. The coated XG beads prepared with a high concentration of Eudragit show high density of Eudragit on the core beads, resulting in a low drug release rate. The rate of drug release from gel-forming polymers was reported to be controlled by competition between penetration of water into the polymer matrix bead and diffusion of dissolved drug through the gel-formed layer (Bamba *et al.*, 1979). Consequently, when the XG core beads were coated with high concentration of Eudragit, diffusion of the drug molecules through the hydrated polymer layer was retarded, leading to a more sustained drug release.

Fig. 5 shows the effect of enteric coating on the *in vitro* release of indomethacin from XG beads under simulated gastro-intestinal transit condition. The release profile of indomethacin from Eudragit L 100-coated XG beads is compared with that from the uncoated ones. The release media were changed after 2 h from simulated gastric fluid at pH 1.2 to a simulated intestinal fluid at pH 7.4 to mimic gastro-intestinal transit. As expected, XG beads coated with an enteric polymer, Eudragit L 100, showed very small amount of drug release, about 3% per hour, when tested under gastric condition below the solubility pH of the Eudragit. Subsequently, remarkable increase in the amount of drug release, about 40% during the first 1 h, was observed, when tested under intestinal condition above the solubility pH of the Eudragit. The pH sensitivity of this polymer is caused by the presence of ionisable carboxyl groups in its molecular structure. The results demonstrated that the enteric-coated beads provide a system of low permeability and a good barrier against drug diffusion under pH conditions, at which protection is required.

In the case of uncoated XG beads, although suppressed under highly acidic condition due to the low solubility of the non-ionized form of indomethacin, which exists at this pH (pK_a of indomethacin, 4.5), and the consequent partitioning of the drug into the polysaccharide chains of

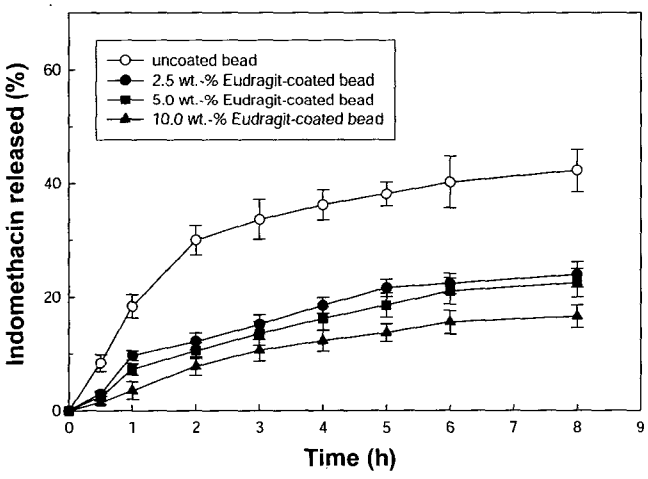

Fig. 4. Effect of Eudragit-coating on the release of indomethacin from xyloglucan beads (XGID 50) in pH 1.2 buffer solution at 37°C. Each point represents Mean±S.D. (n=3).

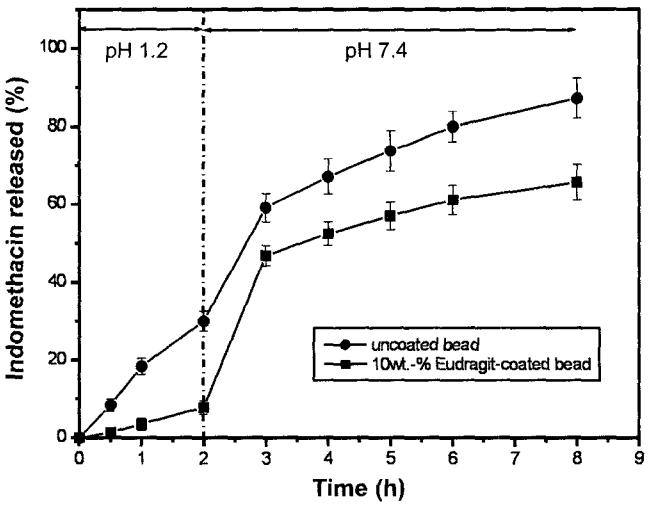

Fig. 5. Effect of enteric coating on the release of indomethacin from xyloglucan beads (XGID 50) under simulated gastro-intestinal transit condition. Each point represents Mean±S.D. (n=3).

Table III. Adhesive forces of polymeric films measured with plastic plate

Condition	Polymer	Adhesive force(N) av±S.D.
Wet state	Carbopol 943 P	0.85±0.05
	Xyloglucan	1.70±0.15
Dry state	Carbopol 943 P	16.9 ±2.9
	Xyloglucan	non-measurable

The data prepresent Mean±S.D. (n=3).

the gels, a more significant release of indomethacin occurred in comparison with Eudragit-coated XG beads (Kawasaki *et al.*, 1999). However, at pH 7.4, indomethacin was fully ionized and had a greater tendency to dissolve into the release medium. The higher release from the uncoated XG beads reflects the lower diffusional resistance of these core beads compared with that of the coated beads caused by the absence of a barrier against drug diffusion.

Adhesive force

Table III shows the adhesive forces of XG and Carbopol 934 P films in dry and wet states. The adhesive force was obtained by measuring the force required to break contact between the XG film and the plastic plate. In wet state, the adhesive force of XG film was stronger than Carbopol 943 P film, a well-known commercial mucoadhesive polymer, whereas, in dry state, could not be measured, because the XG film detached from the plastic plate during the process of drying. Nevertheless, we believe these results are worth notice, considering the wet state of intestines *in vivo*. The results suggest that mucoadhesive XG beads adhere to intestine mucus, thereby prolonging their migration time and extending drug release.

CONCLUSION

The bead type of XG gels to utilize XG as a carrier for oral drug delivery of irritant drug in the stomach was prepared through thermally reversible sol-gel transition, and enteric-coating with Eudragit L 100 was performed. The XG beads were well formed with spherical shape, although the spherical shape in wet state slightly deformed after drying. The effect of drug content on the release of indomethacin from the XG beads differed depending on the medium pH. The release of indomethacin from the XG beads was retarded by coating with Eudragit L 100, and higher retardation observed with the increase in coating concentration and decrease in medium pH. In addition, the drug release from Eudragit-coated XG beads increased rapidly with the change in medium pH from 1.2 to 7.4. Furthermore, the adhesive force of XG was stronger than that of Carbopol 943 P in wet state. Results of our study suggest that this enteric-coated XG beads may be suitable as a carrier for the anti-inflammatory drugs, which are known to cause such side effects as irritation of gastric mucosal tissue.

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