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In situ gelling xyloglucan/alginate liquid formulation for oral sustained drug delivery to dysphagic patients

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Abstract

Background: The oral administration of liquid dosage forms of suitable consistency and with sustained release characteristics may provide a means of improving the compliance of geriatric patients who experience difficulties in swallowing conventional solid dosage forms. *Aim*: We have designed and evaluated liquid preparations for administration to dysphagic patients, composed of aqueous mixtures of xyloglucan, which has thermally reversible gelation characteristics, and sodium alginate, which has ion-responsive gelation characteristics. *Method*: The gelation and in vitro and in vivo release characteristics of liquid formulations containing appropriate concentrations of xyloglucan and sodium alginate with mannuronate/guluronate ratios of either 0.5 or 0.8 were assessed. *Results*: Aqueous mixtures of 1.5% xyloglucan and 0.5% alginate had suitable viscosities for ease of swallowing and appropriate gelation temperatures (~33°C) to ensure in situ gelation following oral administration. The in vitro release of paracetamol at pH 5.0 from gels formed by these formulations and also by a 1.5% xyloglucan solution was diffusion-controlled. Plasma levels of paracetamol after oral administration to gastric-acidity controlled rats (pH 5) of a solution containing 1.5% xyloglucan/0.5% alginate showed that a more sustained release was achieved from the gels formed by the in situ gelation of this formulation compared with that of a 1.5% xyloglucan solution. Visual observation of the contents of the rat stomach after oral administration showed that the inclusion of alginate in the xyloglucan solutions was effective in reducing gel erosion, so sustaining drug release. *Conclusions*: Liquid formulations of xyloglucan and sodium alginate in appropriate proportions are of suitable consistency for ease of administration to dysphagic patients and form gels in situ in the rat stomach capable of sustaining the release of paracetamol over a 6-hour period.

Key words: *Alginate; dysphagia; in situ gelation; oral drug delivery; paracetamol; sustained release; xyloglucan*

Introduction

Patients such as the elderly or very young who experience problems with swallowing conventional solid dosage forms (dysphagia) can benefit from oral administration of liquid formulations of suitable consistency. However, oral preparations suitable for administration to dysphagic patients are generally prepared as gels designed to melt at body temperature for ease of swallowing and are not primarily intended as vehicles for the sustained release of drugs. In this study we have examined the sustained release characteristics of an in situ gelling formulation prepared from mixtures of xyloglucan and sodium alginate.

Xyloglucan is a polysaccharide derived from tamarind seeds that is composed of a (1–4)-β-D-glucan backbone chain, which has (1–6)-α-D-xylose (Xyl) branches that are partially substituted by (1–2)-β-D-galactoxylose (Gal). There are three different structures for the repeating units: heptasaccharide (Glu^4Xyl^3), octasaccharide (Glu^4Xyl^3Gal), and nonasaccharide ($Glu^4Xyl^3Gal^2$) oligomers[1]. Although xyloglucan itself does not form a gel, dilute solutions of xyloglucan, which have been partially degraded by

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β-galactosidase, exhibit a thermally reversible sol-gel transition on heating. The temperature of gel transition is concentration dependent and is also related to the extent of galactose removal, with an optimum of between 35% and 50% for thermally responsive gelation at a suitable temperature range for biomedical application[2]. Thermally reversible xyloglucan formulations have been investigated for as potential vehicles for oral administration[3–6].

Alginate extracted from brown seaweed (*Phaeophyceae*) consists of linear chains of (1–4)-linked β-D-mannuronate (M) and (1–4)-linked α-L-guluronate (G) residues. The residues occur in blocks of homogeneous (MM and GG) or mixed (MG) sequences, and the overall composition and arrangement varies with the source. A dilute solution of alginate forms gels by hydrogen bonding at low pH (acid gel) and by ionic interactions with divalent (Ca^{2+}) or trivalent ions. G-blocks are mainly the responsive domain for ionic interactions with multivalent cations, and they can associate to form aggregates at 'egg-box' junctions[7,8]. The selective binding of divalent metal ions and the corresponding gel strength increase in the order MM-block < MG-block < GG-block[9]. The solution viscosity, molecular weight, and primary structure are fundamental in determining the swelling and gelling properties of alginates. In general terms, alginates containing a high G content develop a stiffer, more brittle, and more porous gels that are heat-stable, whereas high M alginates develop softer and more elastic gels that have less heat stability but more freeze/thaw stability. Numerous applications of calcium alginate gel beads or microspheres for drug delivery systems have been reported[10–12]. We have reported in situ gelling sodium alginate formulation for oral administration[5,13,14].

Morris[15] has observed that an appropriately blended mixture of two or more different polymers can often produce a new system with the desirable features of its constituents. In this respect, we have previously investigated an in situ gelling polysaccharide formulation composed of a blend of xyloglucan, which has thermoresponsive gelling properties, and pectin, which has ion-responsive gelling properties, and reported its potential as a vehicle for oral sustained drug delivery[16]. In a previous study we assessed the suitability of a series of polysaccharide gel formulations for oral administration to dysphagic patients[17]. The aim of this study was to develop an in situ gelling vehicle composed of a mixture of xyloglucan and alginate for oral administration in liquid form to dysphagic patients. The sustained release characteristics of gels formed in situ in the stomachs of rats were determined using gastric-acidity controlled rats at pH 5.0 rather than 1.2 to more realistically represent the lower gastric hydrogen ion concentration in the elderly, which is a consequence of decreased acid secretion[18].

Materials and methods

Materials

Xyloglucan with a percentage of galactose removal of 45% (Lot 3603) was prepared as described previously[2] and supplied by Dainippon Sumitomo Pharma Co., Ltd. (Osaka, Japan). Two grades of sodium alginate, Duck Algin® 350G (alginate G, M/G ratio 0.5) and Duck Algin® 350M (alginate M, M/G ratio 0.8) were obtained from Food Chemifa Co., Ltd. (Tokyo, Japan). Paracetamol (acetaminophen) was obtained from Astellas Pharma Inc. (Tokyo, Japan). Brilliant blue was obtained from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). Ultra pure water was prepared by Barnstead NANOpure® UV (Thermo Fisher Scientific Inc., Waltham, MA, USA). All other reagents were of analytical grade.

Preparation of polysaccharide solutions

The xyloglucan and xyloglucan/alginate solutions were prepared by dispersing the required amount of the xyloglucan and alginate in ultra pure water at 65–70°C. The resulting combination was thoroughly mixed using a homogenizer (CM-200; AS ONE Corp., Osaka, Japan) at 2000 rpm for 10 minutes at room temperature, a further 10 minutes at 2000 rpm with ice cooling, and finally for 50 minutes at 3000 rpm with ice-cooling. The alginate solution was prepared by dispersing the required amount of alginate in ultra pure water and heating to 60°C with stirring. To prepare the polysaccharide solutions containing paracetamol, the desired amounts of paracetamol were added when the xyloglucan and alginate were dispersed. Gel compositions and solution concentrations are expressed as % w/w.

Determination of gelation temperature

Gelation temperatures of xyloglucan/alginate solutions (10 mL) containing amounts of alginate over the range 0–1.0% were measured using a sine-wave vibro viscometer (SV-10; A&D Co., Ltd., Tokyo, Japan) equipped with a water jacket through which water from a low-temperature bath (NCB-1200; Tokyo Rikakikai Co., Ltd., Tokyo, Japan) was circulated. The gelation temperature was identified as an inflection point in the viscosity–temperature curve of the sample solutions corresponding to the abrupt increase of viscosity at the onset of gelation.

Measurement of viscosity of xyloglucan/alginate solutions

The viscosities of the xyloglucan and xyloglucan/alginate solutions were determined at 5°C with a cone

and plate-type rotational viscometer with a cone angle of 1°34′ (TV-20H, model E; Tokyo Keiki Inc., Tokyo, Japan) using 1-mL aliquots of the sample. Measurements on each sol were performed in triplicate over a shear rate range of approximately 23–230 s^{-1}, each shear rate sweep taking approximately 30 seconds.

Measurement of in vitro drug release

Measurement of the in vitro release of paracetamol from xyloglucan, alginate, and xyloglucan/alginate solutions was carried out using plastic dialysis cells similar to that described previously[19]. The capacity of each half-cell was 4 mL and the surface area of the membranes was 2.67 cm^2. Formulations containing 1.0% paracetamol were placed in the donor compartment, and an equal volume of simulated gastric fluid as specified for the JP XV disintegration test was adjusted to pH 5.0 and was placed in the receptor compartment. The gel donor phase and the aqueous receptor phase were separated by a dialysis membrane (Viskase® Co., Inc., Darien, IL, USA). The assembled cell was shaken horizontally at the rate of 60 strokes/min in an incubator at 37°C. The total volume of the receptor solution was removed at intervals and replaced by fresh release medium. The concentration of paracetamol in the samples was determined from the absorbance at a wavelength of 244 nm using a spectrophotometer (UV-1200; Shimadzu Co., Kyoto, Japan).

In vivo drug release

Male Wistar rats, weighing 220–320 g, provided by Hokudo Co., Ltd. (Sapporo, Japan), were fasted for 24 hours with free access to water. The gastric acidity was controlled at pH 5.0 by i.p. injection of 30 mg/0.5 mL of famotidine solution, prepared by diluting 0.3 mL of Gaster® (Astellas Pharma Inc.) with 0.2 mL saline, and the rats were then used for the in vivo experiments after 30 minutes. The intragastric pH was measured directly on the surface of stomachs removed from anesthetized (urethane 1 g/kg by i.p. injection) rats, using a pH composite electrode for surface measurement (SE-1600GC; Chemical Instruments Co. Ltd., Tokyo, Japan) attached to a pH meter (F-22; Horiba Ltd., Tokyo, Japan).

The gastric-acidity controlled rats were anesthetized (urethane 1 g/kg by i.p. injection), and the jugular vein was cannulated to facilitate removal of blood samples. One milliliter samples of 1.5% xyloglucan, and 1.5% xyloglucan/0.5% alginate solutions containing 10 mg paracetamol were orally administrated using a stomach sonde needle for rats (KN-349D; Natsume Seisakusho Co., Ltd., Tokyo, Japan). At given intervals, a blood sample was taken from the jugular vein and analyzed as described below. The protocols for the animal experiments were previously approved by the Animal Ethics and Research Committee of the Health Sciences University of Hokkaido.

The statistical significance of the results was assessed by the Student's *t*-test and results are presented as the mean ± SE.

Determination of paracetamol concentration in rat plasma

The plasma samples were separated by centrifugation and assayed by high-performance liquid chromatography. The high-performance liquid chromatographic system consisted of a pump (LC-10AS; Shimadzu Co.), a UV detector (SPD-10A; Shimadzu), and a data processor for chromatography (C-R6A Chromatopac; Shimadzu). The elution peak was detected at a wavelength of 254 nm.

The assay of paracetamol was based on the methods described by Ameer et al.[20] with minor modifications. To 100 µL of plasma was added 200 µL of water, 100 µL of 2-acetamidophenol (100 µg/mL in 20% methanol) as internal standard, and 7 mL of ethyl acetate. The sample was shaken and centrifuged. Five milliliter of the ethyl acetate layer was evaporated to dryness under a nitrogen stream at 40°C. The residue was reconstituted with 200 µL of 50% methanol and an aliquot of 20 µL was injected onto an analytical column (150 × 4.6 mm i.d.), packed with Inertsil-ODS (GL Sciences Inc., Tokyo, Japan). Elution was carried out with acetonitrile—0.1 M acetate buffer (pH 4.0) (15:85) at a rate of 0.8 mL/min at 40°C.

Visualization of gels in rat stomach

One milliliter samples of 1.5% xyloglucan and 1.5% xyloglucan/0.5% alginate solutions containing 0.02% brilliant blue as a marker dye but no drug were orally administered to fasted rats as described above. Stomachs were excised after 0.5 and 5 hours and gels were removed and weighed after removing surface dirt.

Results and discussion

Gelation of xyloglucan/alginate mixtures

Figure 1 shows the effect of alginate concentration on the gelation temperature of xyloglucan/alginate solutions. 1.0% and 1.5% xyloglucan solutions gelled at 33.6 ± 0.1°C and 32.9 ± 0.7°C ($n = 5$, mean ± SE), respectively. Addition of alginate G (M/G ratio 0.5) at concentrations of between 0.25% and 0.75% to solutions containing 1.0% xyloglucan increased the gelation temperature to 36.4–36.8°C; no gelation was observed in the presence of

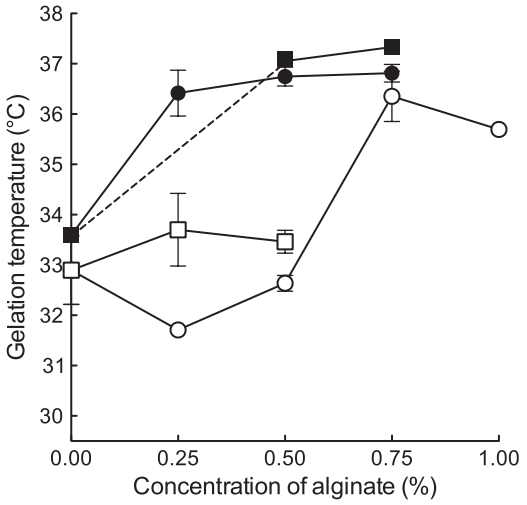

Figure 1. Effect of alginate concentration on the gelation temperature of xyloglucan/alginate mixture solutions. (●) 1.0% xyloglucan/alginate G, (■) 1.0% xyloglucan/alginate M, (○) 1.5% xyloglucan/alginate G, and (□) 1.5% xyloglucan/alginate M. Each value is the mean ± SE of five determinations.

1.0% alginate G. The gelation temperature of solutions of 1.0% xyloglucan was increased to approximately 37.2°C in the presence of 0.5% and 0.75% alginate M (M/G ratio 0.8) but gelation was not observed in the presence of 0.25% or 1.0% of this alginate.

For solutions containing 1.5% xyloglucan, addition of alginate M had insignificant effect on the gelation temperature at concentrations of 0.25% and 0.50% and inhibited gelation at higher concentrations. In contrast, alginate G increased gelation temperature to about 35.7–36.4°C at alginate concentrations of 0.75% and 1.0%.

These results show the sensitivity of gelation temperature to the presence of alginate and in particular the inhibiting effect of both low and high alginate concentrations on gelation. Ideally the gelation temperature should exceed room temperature so that the formulation can be administered in liquid form but should not exceed body temperature to ensure that gelation occurs in the stomach. On this basis, it is suggested that a 1.5% xyloglucan solution containing either 0.25% or 0.5% of alginate G or M had the most suitable gelation temperatures for use as an in situ gelling liquid formulation.

Observations were also made of the ion-responsive gel-forming properties of solutions of 1.5% xyloglucan and 1.5% xyloglucan/alginate (G and M) mixtures when poured into simulated gastric fluid at 37°C. The 1.5% xyloglucan solution formed a soft gel after several minutes, which because of the time taken for gelation, had a poorly defined shape. In contrast, 1.5% xyloglucan solutions containing 0.25% or 0.5% alginate (both G and M) formed gels immediately on contact with the simulated gastric juice.

Viscosity of xyloglucan/alginate solutions

Figure 2 shows the shear dependency of the viscosity at 5°C of 1.5% xyloglucan solution, and 1.5% xyloglucan/alginate mixtures with alginate (G and M) concentrations of between 0.25% and 0.75%. All solutions showed shear-thinning non-Newtonian flow characteristics. It is interesting to note that although solutions of sodium alginate at these concentrations had viscosities below 50 mPa·s at shear rates of between 23 and 230 s^{-1} (data not shown in Figure 2), nevertheless the addition of these concentrations of sodium alginate to the xyloglucan solutions caused a marked increase of viscosity suggesting significant interaction between the two polysaccharides. Although it is generally difficult for dysphagic patients to take low viscosity liquids, too great an increase in viscosity can prevent aspiration by inhibiting the bolus dispersion. On this basis, the 1.5% xyloglucan/0.5% alginate mixtures, which have flow characteristics resembling those of 'runny honey', were considered to be the most suitable for administration to dysphagic patients.

In vitro drug release studies

The release profiles of paracetamol from gels formed from solutions of 0.5% alginate (G and M), 1.5% xyloglucan, and 1.5% xyloglucan/0.5% alginate (G and M) mixtures are compared in Figure 3. The receptor solutions of the diffusion cell were maintained at pH 5.0 during the release period to correspond to the in vivo release experiments described below. The pK_a of paracetamol is 9.5[21], and consequently this drug will be in the fully ionized form at pH 5.0.

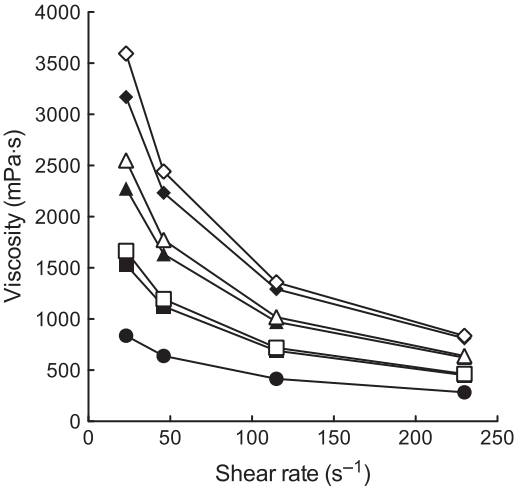

Figure 2. Shear rate dependence of the viscosity at 5°C of (●) 1.5% xyloglucan and 1.5% xyloglucan/alginate solutions containing (■) 0.25% alginate M, (□) 0.25% alginate G, (▲) 0.5% alginate M, (△) 0.5% alginate G, (◆) 0.75% alginate M, and (◇) 0.75% alginate G. Each value is the mean ± SE of three determinations.

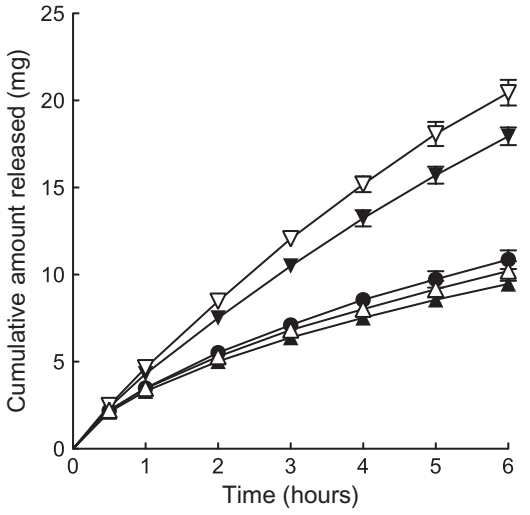

Figure 3. In vitro release of paracetamol from xyloglucan, alginate, and xyloglucan/alginate solutions plotted as cumulative release against time at 37°C. (●) 1.5% xyloglucan, (▼) 0.5% alginate M, (▽) 0.5% alginate G, (▲) 1.5% xyloglucan/0.5% alginate M, and (△) 1.5% xyloglucan/0.5% alginate G. Each value is the mean ± SE of four determinations.

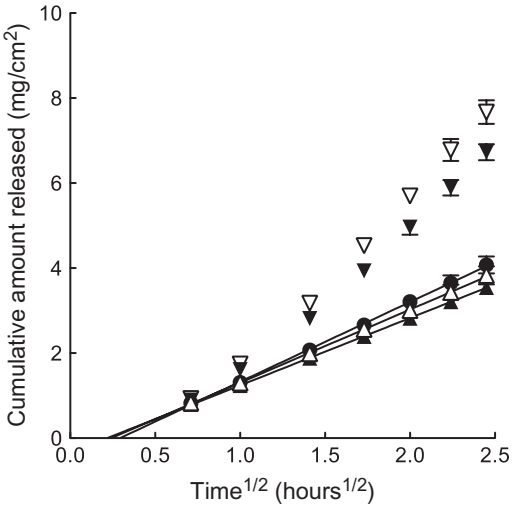

Figure 4. In vitro release of paracetamol from xyloglucan, alginate, and xyloglucan/alginate mixture solutions plotted as cumulative release against square root time at 37°C. (●) 1.5% xyloglucan, (▼) 0.5% alginate M, (▽) 0.5% alginate G, (▲) 1.5% xyloglucan/0.5% alginate M, and (△) 1.5% xyloglucan/0.5% alginate G. Each value is the mean ± SE of four determinations.

Alginate gels form by cross-linking in the presence of ions, usually divalent ions such as Ca^{2+}. Much weaker gels may, however, be formed in sufficiently high concentrations of monovalent ions, particularly H^+. The rapid release from the 0.5% alginate (G and M) formulations observed in Figure 3 suggests that the hydrogen ion concentration at pH 5.0 was too low to induce structured gel formation. This was confirmed by observation of the donor cell.

The release profiles of the gels of 1.5% xyloglucan and 1.5% xyloglucan/alginate (G and M) mixtures indicated a much slower release compared with the alginate gels and observation of the donor cells after 6 hours showed that these gels had retained their structure.

The release data over the whole time of the release for the gel-forming formulations were analyzed using the Higuchi equation for drug release from semisolid vehicles containing dissolved drug,

$$Q = 2C_0\left(\frac{Dt}{\pi}\right)^{1/2} \quad (1)$$

where Q is the cumulative amount of drug released per unit surface area, C_0 is the initial drug concentration, and t is the time[22]. Plots of Q versus $t^{1/2}$ for the release of paracetamol from the gels formed by xyloglucan and xyloglucan/alginate mixtures are shown in Figure 4. Release conformed to Equation (1) after a short lag period indicating a diffusion-controlled process. The diffusion coefficients, D, calculated from the gradients of Higuchi plots of Figure 4 are shown in Table 1; there is a tendency for a decrease in D as a consequence of the inclusion of alginate into the xyloglucan gels, suggesting the formation of more structured gels.

Table 1. Diffusion coefficients, D, for in vitro release of paracetamol from xyloglucan and xyloglucan/alginate gels at pH 5.0.

Formulation	$10^6\,D$ (cm^2/s)
1.5% Xyloglucan	7.77 ± 0.96
1.5% Xyloglucan/0.5% alginate G	6.55 ± 0.82
1.5% Xyloglucan/0.5% alginate M	5.51 ± 0.34

Each value is the mean ± SE of four determinations.

In vivo drug release studies

Plasma drug levels following oral administration to gastric-acidity controlled rats (pH 5.0) of paracetamol (10 mg/1 mL) from solutions of 1.5% xyloglucan, and 1.5% xyloglucan/0.5% alginate (G and M) mixtures, are compared in Figure 5. No results are given for the 0.5% alginate formulations that did not form gels at this pH.

The more sustained release from gels formed by xyloglucan/alginate mixtures compared to the xyloglucan gel was in agreement with the lower in vitro diffusion coefficients observed following the incorporation of alginate into the xyloglucan gels. In particular, the plasma concentration was maintained within a narrow range of between approximately 1.8 and 2.5 µg/mL during the initial 4 hours of release from the xyloglucan/alginate G formulation.

Table 2. Pharmacokinetic parameters of paracetamol administrated from xyloglucan and xyloglucan/alginate gels formed in situ in rat stomach at pH 5.0.

Formulation	C_{max} (μg/mL)	t_{max} (hours)	$AUC_{(0-6\ hours)}$ (μg·h/mL)	MRT (hours)
1.5% Xyloglucan	5.81 ± 1.16	1.63 ± 0.55	11.23 ± 1.06	2.20 ± 0.25
1.5% Xyloglucan/0.5% alginate G	2.74 ± 0.44*	2.88 ± 0.78	11.07 ± 1.43	2.86 ± 0.07*
1.5% Xyloglucan/0.5% alginate M	3.04 ± 0.39	2.00 ± 0.05	9.81 ± 0.71	2.65 ± 0.15

Each value is the mean ± SE of three or four determinations.
*$P < 0.05$ compared with 1.5% xyloglucan.

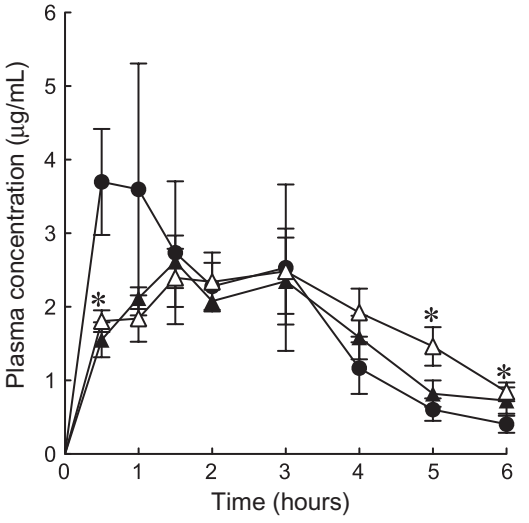

Figure 5. Plasma concentrations of paracetamol after oral administration of (●) 1.5% xyloglucan, (△) 1.5% xyloglucan/0.5% alginate G, and (▲) 1.5% xyloglucan/0.5% alginate M. Each value is the mean ± SE of three or four determinations. *$P < 0.05$ compared with 1.5% xyloglucan.

Pharmacokinetic parameters, the area under the concentration curve (AUC), and the mean residence time (MRT) obtained from the plasma concentration–time data of each animal using a computer program for model-independent analysis[23] are compared in Table 2. The more sustained release from gels formed from the 1.5% xyloglucan/0.5% alginate G solution is evident from the higher MRT value for this mixture.

The appearance of the gels formed in the rat stomach at pH 5.0 following administration of 1-mL solutions of 1.5% xyloglucan and 1.5% xyloglucan/0.5% alginate G is compared in Figure 6. The enhancement of the rigidity of the gels and the consequent slower erosion caused by the incorporation of alginate G is very clear, with 88% of the amount of the mixed gel initially administered remaining after 5 hours compared with only 27% of the gel composed of xyloglucan alone. These results suggest that the xyloglucan/alginate G solution is able to form a rigid gel in situ in the stomachs of rats at pH 5.0, which is able to resist erosion and sustain the release of paracetamol at a low and constant level over a period of about 6 hours after oral administration.

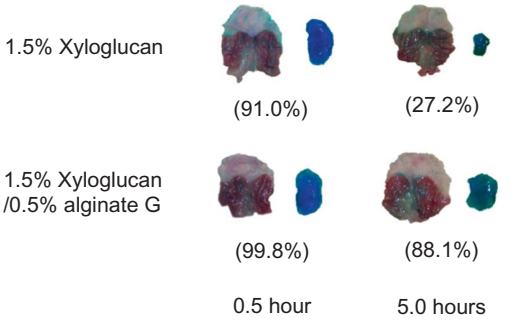

Figure 6. Photographs showing presence of gels in rat stomach (pH 5.0), 0.5 and 5.0 hours after oral administration of 1.5% xyloglucan and 1.5% xyloglucan/0.5% alginate G. Numbers in parentheses indicate the percentage of gel remaining.

Conclusions

This study has demonstrated the potential of mixtures of the polysaccharides xyloglucan and sodium alginate for use in the development of vehicles with thermo/ion-responsive gelation properties suitable for in situ gel formation following oral administration as a liquid formulation. A consideration of the desirable characteristics suggests that a solution of 1.5% xyloglucan and 0.5% G-rich grade (M/G ratio 0.5) alginate has a suitable viscosity for oral administration to dysphagic patients and forms a rigid gel in the rat stomach at pH 5.0, which sustains the release of paracetamol over a 6-hour period.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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