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# In situ gelling xyloglucan/pectin formulations for oral sustained drug delivery

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**Abstract**

This study has examined the gelation and release characteristics of mixtures of xyloglucan, which has thermally reversible gelation characteristics, and pectin, the gelation of which is ion responsive, with the aim of formulating an in situ gelling vehicle suitable for oral sustained drug delivery. An investigation of the effect of the inclusion of pectin (0.75% (w/w)) on the rheological properties of gels formed from solutions of xyloglucan (1.5 and 2.0% (w/w)) showed a significantly greater gel strength when pectin was present in the formulation. The in vitro release of paracetamol from gels containing 1.5% (w/w) xyloglucan, and 1.5 or 2.0% (w/w) xyloglucan/0.75% (w/w) pectin was diffusion-controlled. Measurement of plasma levels of paracetamol after oral administration to rats of a solution containing 1.5% (w/w) xyloglucan and 0.75% (w/w) pectin showed that a more sustained release and higher drug bioavailability was achieved from the gels formed by the in situ gelation of this formulation compared to that of a 1.5% (w/w) xyloglucan solution; 0.75% (w/w) solutions of pectin did not form gels under these conditions. Visual observation of the contents of the rat stomach at intervals after oral administration showed that the inclusion of pectin in the xyloglucan solutions was effective in reducing gel erosion, so sustaining drug release.


*Keywords:* Xyloglucan; Pectin; In situ gelation; Sustained release; Oral drug delivery; Paracetamol

## 1. Introduction

In situ gel forming systems have been widely investigated as vehicles for sustained drug delivery. In particular, thermally responsive hydrogels, which show sol–gel transition at body temperature are of interest in biomedical applications, including drug delivery (Ruel-Gariépy and Leroux, 2004). Other factors influencing in vivo gelation, in addition to temperature change, include the high gastric acidity and the presence of metal ions in the gastric juice. Recently, hydrogels have been formulated from mixtures of polymers with thermal and ion-responsive gelation characteristics. For example, Lin et al. (2004) have investigated the application of such mixtures for ocular drug delivery in order to reduce total polymer content and improve gelling properties. An additional advantage of the use of mixtures of polysaccharides is that under favourable circumstances there may be a synergism between the component polymers resulting in beneficial changes in their properties (Morris, 1995).

In this study we have prepared polysaccharide-based hydrogels containing xyloglucan, which has thermally reversible gelling properties, and pectin, the gelation of which is ion responsive. Xyloglucan is a polysaccharide derived from tamarind seeds and 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. Xyloglucan is composed of heptasaccharide, octasaccharide and nonasaccharide oligamers, which differ in the number of galactose side chains as shown in Scheme 1 (York et al., 1990). Although xyloglucan itself does not gel, dilute solutions of xyloglucan which has been partially degraded by β-galactosidase exhibit a thermally

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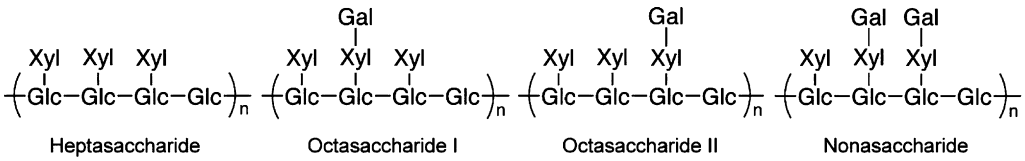


Scheme 1. Backbone structure of xyloglucan. Glc, Xyl and Gal indicate β-D-glucopyranosyl, β-D-xylopyranosyl and β-D-galactopyranosyl residues, respectively.

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 (Shirakawa et al., 1998). For example, aqueous solution of xyloglucan with a percentage of galactose removal of 44%, gel at temperatures between 27 and 22 °C in dilute aqueous solutions over the concentration range 1.0 and 2.0% (w/w) (Miyazaki et al., 1998). Thermally reversible xyloglucan formulations have been investigated for rectal (Miyazaki et al., 1998), intraperitoneal (Suisha et al., 1998), ophthalmic (Burgalassi et al., 2000; Miyazaki et al., 2001a), percutaneous (Takahashi et al., 2002), and oral (Kawasaki et al., 1999; Miyazaki et al., 2001b, 2003) administrations.

Pectins are anionic polysaccharides extracted from cell walls of most plants, and are composed of a backbone of poly-(1-4)-α-D-galacturonic acid. Pectins are commercially available as low methoxy (LM) pectin (degree of esterification (DE) < 50%) and high methoxy (HM) pectin (DE > 50%) (Thakur et al., 1997). LM pectins form a gel in the presence of divalent ions such as $Ca^{2+}$, and can also gel in the absence of $Ca^{2+}$ when the pH is below about 3.3 (Gilsenan et al., 2000). We have reported the potential of in situ gelling pectin gels for sustained drug delivery (Kubo et al., 2004a,b, 2005; Miyazaki et al., 2005; Itoh et al., 2006a,b, 2007).

In the present investigation we have explored the potential of an in situ gelling xyloglucan/pectin mixture for the oral administration of paracetamol. The formulations have been optimized to produce gelling and rheological properties suitable for oral administration and the in vitro/in vivo drug release characteristics have been examined.

## 2. Materials and methods

### 2.1. Materials

Xyloglucan with a percentage of galactose removal of 45% (Lot. 3603) was prepared as described previously (Shirakawa et al., 1998) and supplied by Dainippon Sumitomo Pharma Co., Ltd. (Osaka, Japan). Pectin (LM-104-ASJ, DE = 31%, Lot. 23001-7) was supplied by SANSHO Co., Ltd. (Osaka, Japan). Paracetamol (acetaminophen) was obtained from Astellas Pharma Inc. (Tokyo, Japan). All other reagents were of analytical grade.

### 2.2. Preparation of solutions

The xyloglucan and xyloglucan/pectin solutions were prepared by dispersing the required amount of the polysaccharide or polysaccharide mixture in ultra pure water (NANOpure® Diamond™ UF and UV/UF Systems, Barnstead International, IA, U.S.A.) at 65–70 °C. The resulting combination was mixed completely using a homogenizer (CM-200, AS ONE Corp., Osaka, Japan) at 2000 rpm for about 10 min at room temperature, a further 10 min at 2000 rpm with ice-cooling and finally 50 min at 3000 rpm with ice-cooling. The solution of pectin was prepared by dispersing the required amount of pectin in ultra pure water and heating to 40–50 °C with stirring. To prepare the polymer solutions containing paracetamol, the desired amounts of paracetamol were added when the xyloglucan and pectin were dispersed.

### 2.3. Determination of flow behaviour of sols

Sample tubes containing 10 ml of polymer solutions initially at 2 °C were incubated for 10 min at 37 °C in a water bath and their flow properties observed visually. Gel formation was indicated by a lack of movement of the meniscus on tilting the tube.

### 2.4. Measurement of rheological properties of sols and gels

The viscosities of the xyloglucan solution and the xyloglucan/pectin mixtures were determined at 5 °C with a cone and plate viscometer with a cone angle of 1°34′ (TV-20H, model E, Toki Sangyo Co., Ltd., Tokyo, Japan) using 1 ml aliquot 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 s.

The influence of pectin content on the gel strengths of the xyloglucan/pectin mixtures was measured using a rheometer (CR-500DX, Sun Scientific Co., Tokyo, Japan) by the method described previously (Watanabe et al., 1994). Cylindrical gels of the polymer solutions were prepared by placing 30 ml of the sol into cellulose tubing (size 36/32, Viskase Companies Inc., IL, U.S.A.), immersing the tube in 150 ml of pH 1.2 simulated gastric fluid (as specified for the JP XV disintegration test) and equilibrating for 24 h at 37 °C. The cylindrical gels (15 mm diameter and 15 mm height) were placed in the rheometer and raised at a rate of 60 mm $min^{-1}$ so pushing a probe slowly through the gel. The change in the load on the probe was measured as a function of the depth of immersion of the probe below the gel surface.

### 2.5. Measurement of in vitro drug release

Measurement of the in vitro release of paracetamol from xyloglucan, pectin and xyloglucan/pectin solutions was carried

out using plastic dialysis cells similar to that described previously (Miyazaki et al., 1984). The capacity of each half-cell was 4 ml and the surface area of the membranes was 2.67 $cm^2$. Polymer solutions containing 1.0% (w/v) paracetamol were placed in the donor compartment, and an equal volume of simulated gastric (pH 1.2) or intestinal (pH 6.8) fluid (as specified for the JP XV disintegration test) was placed in the receptor compartment. The gel donor phase and the aqueous receptor phase were separated by a dialysis membrane (Viskase Companies Inc.). The assembled cell was shaken horizontally at the rate of 60 strokes $min^{-1}$ 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, Kyoto, Japan).

### 2.6. In vivo drug release

Male Wistar rats, weighting 230–330 g, were provided by Hokudo Co., Ltd. (Sapporo, Japan). The rats were fasted for 24 h with free access to water. They were anaesthetised with an intraperitoneal injection of urethane, 1 g $kg^{-1}$, and the jugular vein was cannulated to facilitate removal of blood samples. 1 ml of solutions of 1.5% (w/w) xyloglucan and 1.5% (w/w) xyloglucan/0.75% (w/w) pectin 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 ± standard error of the mean.

### 2.7. Determination of paracetamol concentration in rat plasma

The plasma samples were separated by centrifugation and assayed by HPLC. The HPLC chromatographic system consisted of a pump (LC-10AS, Shimadzu, Kyoto, Japan), with a UV detector (SPD-10A, Shimadzu) at a wavelength of 254 nm.

The assay of paracetamol was based on the methods described by Ameer et al. (1981) with minor modifications. To 100 μl of plasma was added 200 μl of water, 100 μl of 2-acetamidophenol (100 μg $ml^{-1}$ 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. The residue was reconstituted with 200 μl of 50% methanol and an aliquot of 20 μl was injected onto an analytical column (150 mm × 4.6 mm), 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 $min^{-1}$ at 40 °C.

### 2.8. In situ gel forming property in rat stomach

One milliliter of solutions of 1.5% (w/w) xyloglucan and 1.5% (w/w) xyloglucan/0.75% (w/w) pectin mixture containing 0.02% (w/v) brilliant blue as a marker dye but no drug were orally administrated to fasted rats as described previously (Section 2.6). The stomach was excised after 0.5 and 3 h and gels were removed and weighed after removing surface dirt.

## 3. Results and discussion

### 3.1. Gelation of xyloglucan/pectin mixtures

Preliminary observations were made of the thermo-responsive gelling properties of xyloglucan/pectin mixtures to select suitable formulations for further investigation. 1.0, 1.5 and 2.0% (w/w) xyloglucan solutions were mixed with various concentrations of pectin between 0 and 1.25% (w/w) and the flow behaviour assessed visually after 10 min at 37 °C. The 1.0% (w/w) xyloglucan solution formed a soft gel in the absence of added pectin, but failed to gel when pectin was present in the solutions at concentrations greater than 0.25% (w/w). 1.5 and 2.0% (w/w) xyloglucan solutions formed soft gels at pectin concentrations of between 0 and 0.75% (w/w) and a stronger gel in the presence of 0.75% (w/w) pectin; further addition of pectin, however, inhibited gelation. Formulations of 1.5 and 2.0% (w/w) xyloglucan/0.75% (w/w) pectin were selected for further study.

Observations were also made of the gel forming properties of solutions of 1.5% (w/w) xyloglucan, 0.75% (w/w) pectin and 1.5% (w/w) xyloglcan/0.75% (w/w) pectin when poured into simulated gastric acid (pH 1.2) at 37 °C. A soft gel was immediately formed by the 0.75% (w/w) pectin solution. The 1.5% (w/w) xyloglucan solution formed a soft gel after several minutes, which because of the time taken for gelation, had a poorly defined shape. In contrast, the xyloglucan/pectin solution formed a stiff gel immediately on contact with the gastric juice, the enhanced gel forming properties compared to those of solutions of the component polysaccharides being a consequence of a response to both temperature and ions.

### 3.2. Rheological behaviour of the solutions and gels

Fig. 1 shows the shear stress versus shear rate flow curves of 1.0, 1.5 and 2.0% (w/w) xyloglucan solutions with and without added 0.75% (w/w) pectin at 5 °C. All measurements were performed in triplicate with good reproducibility.

All solutions showed shear thinning behaviour and an increase of viscosity as a function of xyloglucan concentration regardless of the amount of pectin present. Although the viscosity of 0.75% (w/w) pectin was very low (below 10 mPa s, with shear rate of between 23 and 230 $s^{-1}$ at 5 °C), its addition to the xyloglucan solutions caused an increase of viscosity, which in the case of a 2.0% (w/w) xyloglucan solution, was appreciable.

Fig. 2 shows the effects of the concentration of pectin on the viscosity at a shear rate of 23 $s^{-1}$ and indicates an optimum pectin concentration of 0.75% (w/w) for the 1.5 and 2.0% (w/w) xyloglucan solutions, in agreement with the preliminary visual

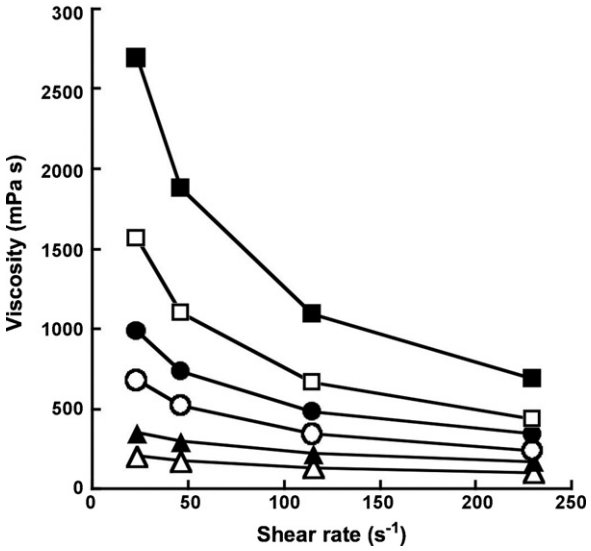


Fig. 1. Viscosity of xyloglucan and xyloglucan/pectin solutions of concentrations (% (w/w)) of 1.0 (triangles), 1.5 (circles), and 2.0 (squares) at 5 °C; closed symbols refer to xyloglucan solution containing 0.75% (w/w) pectin, open symbols refer to xyloglucan solutions without added pectin. Each value is the mean ± S.E. of 3 determinations.

observations. The enhancement of xyloglucan gel strength in the presence of pectin at this concentration is clearly shown by the stress–strain curves of Fig. 3. Curves for gels of both 1.5% (w/w) xyloglucan and a 1.5% (w/w) xyloglucan/0.75% (w/w) pectin mixture showed typical elastic behaviour. Values of the gel strength, taken as the stress at the point of collapse of the gel structure, were 1.7 and 7.8 kN m$^{-2}$ for 1.5% (w/w) xyloglucan and 1.5% (w/w) xyloglucan/0.75% (w/w) pectin, respectively, an increase of approximately 4.6 times in the presence of the pectin.

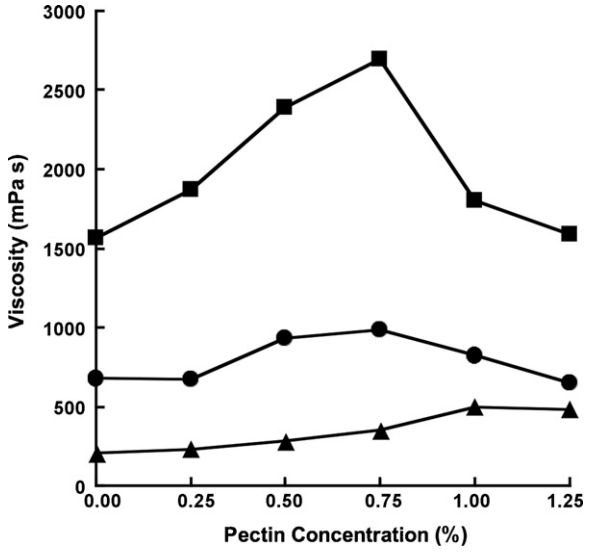


Fig. 2. Effect of added pectin on the viscosity at a shear rate of 23 s$^{-1}$ of xyloglucan solutions of concentrations (% (w/w)), of (▲) 1.0, (●) 1.5, and (■) 2.0 at 5 °C.

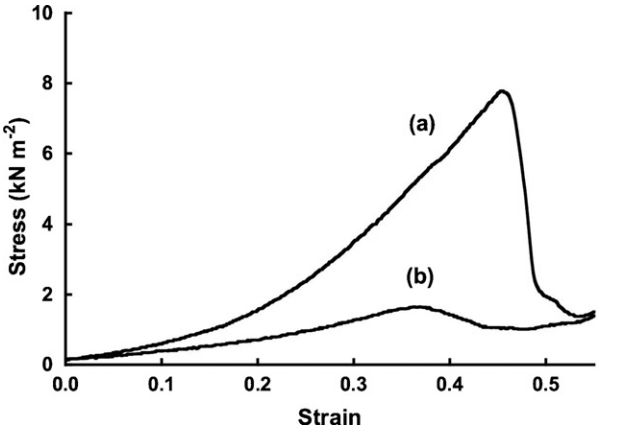


Fig. 3. Stress–strain curve of (a) 1.5% (w/w) xyloglucan gel and (b) 1.5% (w/w) xyloglucan/0.75% (w/w) pectin mixture gel, in simulated gastric fluid at pH 1.2 and 37 °C.

An interaction between xyloglucan and pectin promoting gelation properties has not been reported previously. Nitta et al. (2003) reported that mixtures of tamarind seed xyloglucan (with no removal of galactose residues) and gellan formed thermally reversible gels, although the component polysaccharides alone were unable to gel under the same experimental conditions. The synergistic interaction was explained by Dea et al. (1972) based on a molecular model of interactions between helix-forming polysaccharides and (1-4)-β-D-mannans. They suggested that the galactose-free regions of xyloglucan interact with helical gellan to form a three-dimensional network in the xyloglucan/gellan mixtures. A similar helical model of the gelation of pectinic acid through chain–chain associations has been proposed (Morris et al., 1982; Braccini and Pérez, 2001) and it is probable that the synergistic interaction between xyloglucan and pectin observed in the present study is also explicable using a model of this type.

### 3.3. In vitro drug release

The release profiles of paracetamol from gels formed from solutions of 0.75% (w/w) pectin, 1.5% (w/w) xyloglucan, 1.5% (w/w) xyloglucan/0.75% (w/w) pectin, and 2.0% (w/w) xyloglucan/0.75% (w/w) pectin are compared in Fig. 4. The receptor solutions of the diffusion cell were changed after 1 h from simulated gastric fluid at pH 1.2 to a simulated intestinal fluid at pH 6.8 to mimic gastrointestinal transit. The p$K_a$ of paracetamol is 9.5 (Fairbrother, 1974), and consequently there will be no change in the state of ionization of this acidic drug accompanying this pH change. There was a pronounced increase of release after 2 h from the pectin gel due a reversion to sol as the hydrogen ion concentration was decreased. The release profiles of the gels of xyloglucan and xyloglucan/pectin mixtures were similar and observation of the donor cells after 6 h showed that despite the pH change, all gels had retained their structure.

The release data over the whole time period of the release for these formulations were analysed using the Higuchi equation for drug release from semisolid vehicles containing dissolved drug

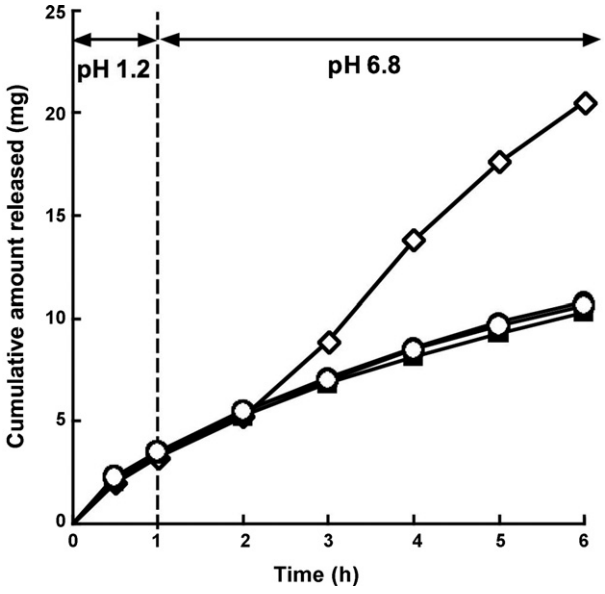


Fig. 4. Cumulative release of paracetamol as a function of time from the gels of (◯) 1.5% (w/w) xyloglucan, (●) 1.5% (w/w) xyloglucan/0.75% (w/w) pectin, (■) 2.0% (w/w) xyloglucan/0.75% (w/w) pectin and (◊) 0.75% (w/w) pectin. Release was into simulated gastric fluid, pH 1.2, for a period of 1 h and subsequently into simulated intestinal fluid, pH 6.8. Each value is the mean ± S.E. of 4 determinations.

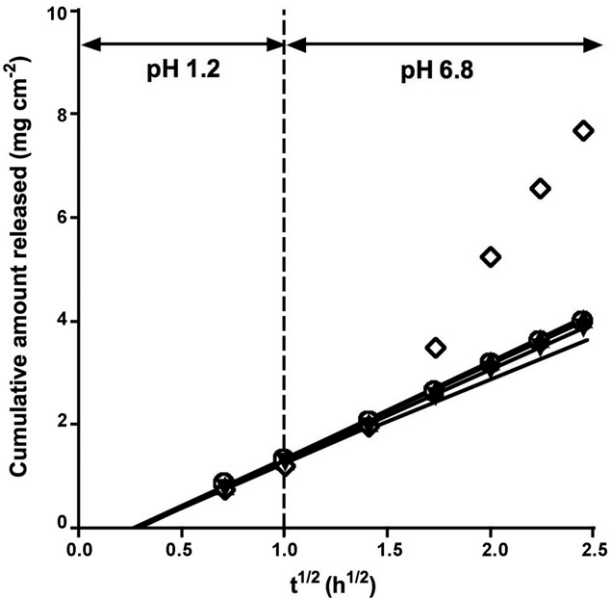


Fig. 5. Cumulative release of paracetamol per unit area, $Q$, as a function of square root of time from the gels of (◯) 1.5% (w/w) xyloglucan, (●) 1.5% (w/w) xyloglucan/0.75% (w/w) pectin, (■) 2.0% (w/w) xyloglucan/0.75% (w/w) pectin and (◊) 0.75% (w/w) pectin. Release was into simulated gastric fluid, pH 1.2, for a period of 1 h and subsequently into simulated intestinal fluid, pH 6.8. Each value is the mean ± S.E. of 4 determinations.

(Higuchi, 1962):

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

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. Plots of $Q$ versus $t^{1/2}$ for the release of paracetamol from the gels are shown in Fig. 5. Release from gels at each of the polymer concentrations conformed to Eq. (1) after a short lag period indicating diffusion-controlled release. The diffusion coefficients, $D$, calculated from the gradients of Higuchi plots of Fig. 5 were $7.28 \times 10^{-6} \pm 0.45$, $7.74 \times 10^{-6} \pm 0.81$ and $6.95 \times 10^{-6} \pm 0.67\ \mathrm{cm^2\ s^{-1}}$ for 1.5% (w/w) xyloglucan, 1.5% (w/w) xyloglucan/0.75% (w/w) pectin, and 2.0% (w/w) xyloglucan/0.75% (w/w) pectin gels, respectively ($n = 4$).

Although in vitro drug release from the xyloglucan and xyloglucan/pectin mixtures was similar, the 2.0% (w/w) xyloglucan/0.75% (w/w) pectin solution had a significantly higher viscosity (Fig. 2), which may be a disadvantage in swallowing the formulation during oral administration. In view of this we chose to conduct in vivo experiments on the 1.5% (w/w) xyloglucan/0.75% (w/w) pectin mixture, which has a satisfactory gel strength and drug release characteristics.

### 3.4. In vivo drug release

The release of paracetamol from gels formed in situ in the rat stomach after oral administration of 1 ml of 0.75% (w/w) pectin solution, 1.5% (w/w) xyloglucan solution and the 1.5% (w/w) xyloglucan/0.75% (w/w) pectin mixture containing 10 mg of paracetamol was monitored by the determination of plasma drug levels (Fig. 6). The mean value of intragastric pH of rat just before administration was 2.58 ± 0.14 ($n = 6$).

Rapid absorption of paracetamol from the pectin solution was observed, with a peak plasma drug concentration of 4.92 μg ml$^{-1}$ at 0.5 h. This concentration of pectin is too low for gel formation under the conditions in the rat stomach (as confirmed by visual observation of rat stomach contents after administration) and hence control of drug release was not

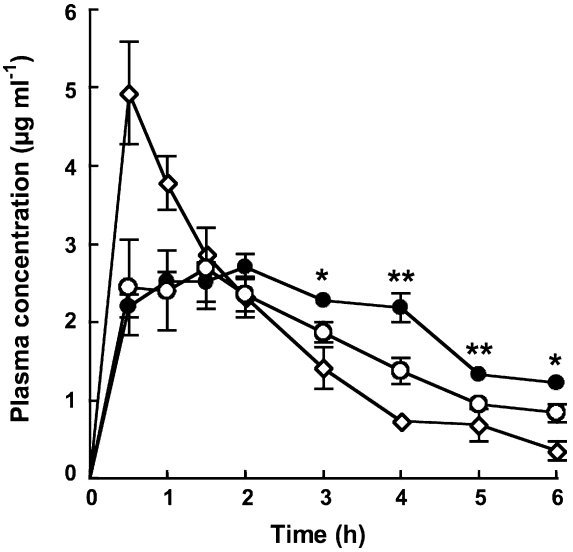


Fig. 6. Plasma concentrations of paracetamol in rats after oral administration of (◊) 0.75% (w/w) pectin, (◯) 1.5% (w/w) xyloglucan, and (●) 1.5% (w/w) xyloglucan/0.75% (w/w) pectin solutions. Each value is the mean ± S.E. of 3–6 determinations. $^{*}p < 0.05$; $^{**}p < 0.01$, compared with 1.5% (w/w) xyloglucan.

Table 1
Comparison of pharmacokinetic parameters of paracetamol administered from aqueous solution and polymer gels formed in situ in rat stomach

| Dosage form | $C_{max}$ (μg ml$^{-1}$) | $t_{max}$ (h) | AUC (0–6 h) (μg h ml$^{-1}$) | MRT (h) |
|---|---|---|---|---|
| 0.75% (w/w) Pectin[a] | 4.92 ± 0.66 | 0.50 ± 0.00 | 10.53 ± 0.20 | 1.93 ± 0.17 |
| 1.5% (w/w) Xyloglucan[b] | 2.98 ± 0.52 | 1.58 ± 0.33 | 10.15 ± 0.70 | 2.58 ± 0.15 |
| 1.5% (w/w) Xyloglucan/0.75% (w/w) pectin[b] | 2.92 ± 0.18 | 2.08 ± 0.20 | 12.05 ± 0.42[c] | 2.79 ± 0.04 |

[a] Each value represents the mean ± S.E. of 3 determinations.
[b] Each value represents the mean ± S.E. of 6 determinations.
[c] $p < 0.05$, compared with 1.5% (w/w) xyloglucan solution.

expected. Drug release from gels formed by xyloglucan and the xyloglucan/pectin mixture gave similar plasma concentrations during the initial 2 h of release, after which time a more sustained release from gels formed from the xyloglucan/pectin mixture was observed.

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 (Yamaoka et al., 1981) are compared in Table 1. The more sustained release from gels formed from the xyloglucan/pectin solution is evident from the higher MRT value compared to that from the xyloglucan solution. Furthermore, the higher AUC value for the mixed system indicates an improved bioavailability from this formulation.

The appearance of the gels formed in the rat stomach following administration of 1 ml of 1.5% (w/w) xyloglucan and 1.5% (w/w) xyloglucan/0.75% (w/w) pectin solutions is compared in Fig. 7. Well-formed gels were observed in the stomach at 0.5 h after administration of both formulations which contained 93–102% of the amount of the dosage administered. At 3 h after administration, approximately 88% of the gel formed from the 1.5% (w/w) xyloglucan/0.75% (w/w) pectin mixture remained in the rat stomach, compared to only 46% of the gel formed from the 1.5% (w/w) xyloglucan solution. At the completion of the release experiments (6 h) the gels were removed and weighed; the amounts of gels remaining were 77.0 ± 4.4% for the xyloglucan/pectin formulation compared to 34.0 ± 13.9% for the xyloglucan formulation ($n = 6$). The significantly slower erosion of the gel formed from the xyloglucan/pectin mixture is a consequence of its much greater gel strength as shown from the in vitro measurements and gives rise to the more sustained release of drug observed in Fig. 6.

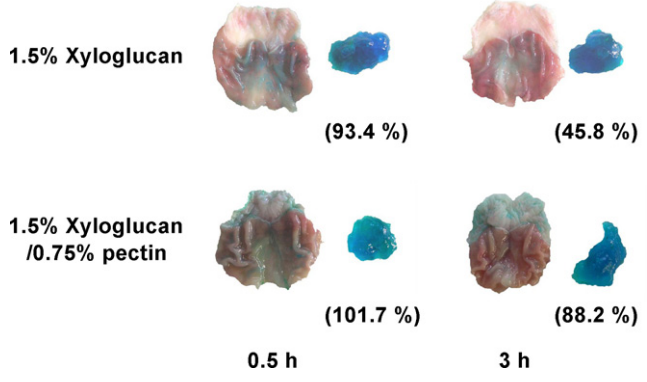


Fig. 7. Photographs showing presence of gels in rat stomach 0.5 and 3 h after oral administration of 1.5% (w/w) xyloglucan solution and 1.5% (w/w) xyloglucan/0.75% (w/w) pectin solution.

## 4. Concluding remarks

Although dilute solutions of xyloglucan will form gels by in situ gelation in the rat stomach when administered orally, this study has shown that the gel strength and sustained release properties may be improved by the inclusion of pectin in the formulation. Improvement of the gelation characteristics of the xyloglucan/pectin mixture is possibly not only a result of a combination of both thermal and ion responsiveness but may also be due to a synergistic interaction between these two polymers.

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