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International Journal of Pharmaceutics 181 (1999) 227–234

international journal of pharmaceutics

Thermally reversible xyloglucan gels as vehicles for oral drug delivery

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Received 10 November 1998; received in revised form 8 January 1999; accepted 18 January 1999

Abstract

The potential, as sustained release vehicles, of gels formed in situ following the oral administration of dilute aqueous solutions of a xyloglucan polysaccharide derived from tamarind seed has been assessed by in vitro and in vivo studies. Aqueous solutions of xyloglucan that had been partially degraded by β-galactosidase to eliminate 44% of galactose residues formed rigid gels at concentrations of 1.0 and 1.5% w/w at 37°C. The in vitro release of indomethacin and diltiazem from the enzyme-degraded xyloglucan gels followed root-time kinetics over a period of 5 h at 37°C at pH 6.8. Plasma concentrations of indomethacin and diltiazem, after oral administration to rats of chilled 1% w/w aqueous solutions of the enzyme-degraded xyloglucan containing dissolved drug, and a suspension of indomethacin of the same concentration were compared. Constant indomethacin plasma concentrations were noted from both formulations after 2 h and were maintained over a period of at least 7 h. Bioavailability of indomethacin from xyloglucan gels formed in situ was increased approximately threefold compared with that from the suspension. The results of this study suggest the potential of the enzyme-degraded xyloglucan gels as vehicles for oral delivery of drugs.

Keywords: Xyloglucan gels; Oral drug delivery; Thermoreversible gels; Sustained release; Indomethacin

1. Introduction

Materials that exhibit sol to gel transition in aqueous solution at temperatures between ambient and body temperature are of interest in the development of sustained release vehicles with in

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0378-5173/99/$ - see front matter
PII: S0378-5173(99)00026-5

situ gelation properties. A compound which has received considerable attention is the polyoxyethylene/polyoxypropylene/polyoxyethylene triblock copolymer Pluronic F127 (poloxamer 407), the thermoreversible gelation of which was demonstrated by Schmolka (1972). Gels of Pluronic F127 have been explored for application in ophthalmic (Miller and Donovan, 1982), topical (Miyazaki et al., 1995, 1984), rectal (Miyazaki et al., 1986; Choi et al., 1998), nasal (Jain et al., 1991), subcutaneous (Morikawa et al., 1987), and intraperitoneal (Miyazaki et al., 1992) administration. There are, however, inherent problems associated with triblock copolymers of polyoxyethylene and polyoxypropylene; commercial samples are subject to batch-to-batch variability (Attwood et al., 1985) and laboratory synthesis is complicated by the so-called transfer reaction, which results in the presence of diblock impurities (Altinok et al., 1997). These problems may be avoided through the use of block copolymers in which oxybutylene is substituted for oxypropylene as the hydrophobe, which can be tailor-made to have the necessary sol–gel transition between ambient and body temperatures to confer in situ gelation characteristics (Tanodekaew et al., 1993). An alternative polymer is the polysaccharide xyloglucan, which also exhibits sol to gel transition in the required temperature region, and which has the additional advantage of recognised non-toxicity and lower gelation concentration (Yuguchi et al., 1997).

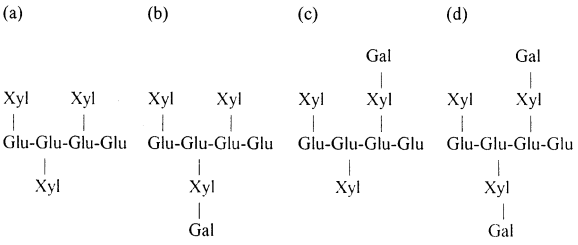
Xyloglucan polysaccharide derived from tamarind seeds is composed of a (1-4)-β-D-glucan backbone chain which has (1-6)-α-D-xylose branches that are partially substituted by (1-2)-β-D-galactoxylose. The tamarind seed xyloglucan is composed of three units of xyloglucan oligomers with heptasaccharide, octasaccharide and nonasaccharide, which differ in the number of galactose side-chains (Scheme 1). When xyloglucan derived from tamarind seed is partially degraded by β-galactosidase, the resultant product exhibits thermally reversible gelation, the sol–gel transition temperature varying with the degree of galactose elimination (Yuguchi et al., 1997). Such gelation does not occur with native xyloglucan. In this study, we have used a xyloglucan sample with a percentage of galactose removal of 44%, which exhibits a transition from sol to gel at temperatures of between 22 and 27°C in dilute aqueous solution over the concentration range 1–2% w/w (Miyazaki et al., 1998). The gelation is thermally reversible, i.e. the gels revert to their sol phase on cooling below the gelation temperature. We previously reported the potential use of xyloglucan gels for rectal (Miyazaki et al., 1998) and intraperitoneal (Suisha et al., 1998) drug delivery, and we now consider the possible application of in situ gelling xyloglucan formulations for oral administration.

2. Materials and methods

2.1. Materials

Galactoxyloglucan with a percentage of galactose removal of 44% was prepared as described previously (Shirakawa et al., 1998). Removal of the required percentage of β-D-galactosidase residues was carried out by reacting a 3% aqueous solution of xyloglucan with a 0.09% aqueous solution of the enzyme β-galactosidase from *Bacilus circulans* (Biolacta N5; Daiwa Kasei K.K., Osaka, Japan). The conditions required for 44% removal are pH 6.0 and 55°C for 16 h. The reaction was terminated at this time by heating to 90°C for 30 min to inactivate the enzyme. The enzyme-degraded xyloglucan was precipitated from this solution by addition of ethanol.

Indomethacin was obtained from Sigma Chemical (St Louis, MO, USA) and diltiazem hydrochloride was supplied by Wako Pure Chemical (Osaka, Japan).



Scheme 1. The unit structures of oligosaccharides from tamarind xyloglucan showing (a) heptasaccharide, (b) and (c) octasaccharides, and (d) nonasaccharide.

Pluronic F127 was a gift from BASF Wyandotte (Parsippany, USA). Sodium alginate, medium viscosity grade, was supplied by Sigma.

2.2. Preparation of sols and suspension

A weighed amount of the enzyme-degraded xyloglucan was slowly added to cold water, pH 1.2 simulated gastric fluid (as specified for the JP XIII disintegration test) or pH 7.4 phosphate buffer. The mixture was slowly homogenized (Nihon Seiki Seisakusho homogenizer type HB) for 1 h at 1,000 rpm. An appropriate amount of indomethacin or diltiazem was then dissolved in the resulting solution.

The control suspension was prepared by adding 0.57% w/v sodium alginate to water and dispersing the indomethacin in the resulting solution.

A 25% w/w sol of Pluronic F127 was prepared by the 'cold method' as described by Schmolka (1972)

2.3. Measurement of gel strength

Measurements were carried out using a rheometer (CR-200D; Sun Scientific, Tokyo) by the method of Watanabe et al. (1994). A 30 g sample of the transparent gel prepared in simulated gastric fluid at pH 1.2 was contained in a 50 ml beaker maintained at constant temperature by a water jacket through which water was circulated at the required temperature from a thermostat bath. The beaker was raised at a rate of 60 mm min^{-1}, so pushing a probe slowly through the gel. The changes in the load on the probe, as a function of depth of immersion of the probe below the gel surface, were measured for gels of concentrations over the range 0.5–1.5% w/w.

2.4. Measurement of drug release rate from gels

The release rates of indomethacin and diltiazem were measured by using a plastic dialysis cell 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. The enzyme- degraded xyloglucan gel, prepared in pH 7.2 buffer and loaded with a known weight of drug, was formed in the donor compartment, and an equal volume of the pH 1.2 simulated gastric fluid was placed in the receptor compartment. The gel donor phase and the aqueous receptor phase were separated by a cellulose membrane (Viskase Sales, size 36/32). The assembled cell was shaken horizontally at the rate of 60 strokes min^{-1} in an incubator. The release medium was replaced by pH 6.8 simulated intestinal fluid after 1 h to simulate passage through the gastrointestinal tract. The total volume of the receptor solution was removed at intervals throughout the release period and replaced by fresh release medium. The concentrations of indomethacin and diltiazem were determined spectrophotometrically at wavelengths of 254 and 237 nm, respectively. All experiments were carried out in triplicate.

2.5. Animal experiments

Male Wistar rats, weighing 200–300 g, were fasted for 24 h with free access to water. The rats were anaesthetised with an i.p. injection of urethane, 1 g kg^{-1}, and the jugular vein was cannulated to facilitate removal of blood sample. Gels containing indomethacin were produced in situ by oral administration of 1 ml of the enzyme-degraded xyloglucan solution containing 1 mg of drug. Administration was by means of a stomach sonde needle for rats (Natume KN-349D) fitted onto a disposable syringe: the sol was cooled prior to filling the syringe to facilitate this procedure. A similar procedure was followed for the administration of 25% w/w sols of Pluronic F127 containing 1 mg of drug. For intravenous administration, 1 mg doses of the drug in 1 ml phosphate buffer at pH 7.4 were injected through the femoral vein. At predetermined intervals, a blood sample was collected from the jugular vein and centrifuged at 3000 rpm for 10 min. The plasma concentration of indomethacin was determined chromatographically by the method of Skellern and Salole (1975) with slight modifications (Miyazaki et al., 1986).

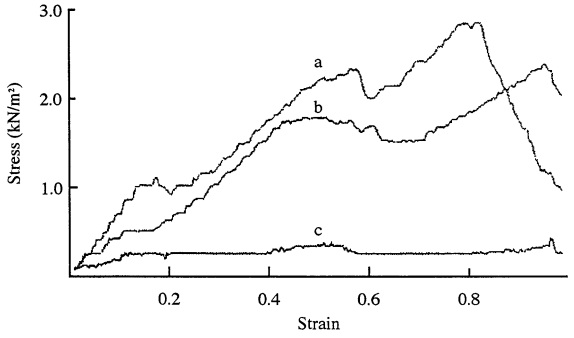


Fig. 1. Rheological properties of xyloglucan gels of concentrations (a) 1.5, (b) 1.0 and (c) 0.5% w/w in simulated gastric fluid, pH 1.2, at 37°C.

3. Results and discussion

3.1. Gelling properties

The gelation properties in simulated gastric fluid, pH 1.2 (as specified for the JP XIII disintegration test), were determined at 25 and 37°C, at concentrations of 0.5, 1.0 and 1.5% w/w. Gel formation was observed in all systems at 37°C, but only with the 1.0 and 1.5% w/w solutions at 25°C. The 0.5% w/w solution formed a viscous solution under these conditions, which exhibited flow on tilting and hence was not classed as a gel according to the definition used in this investigation.

The visual assessment of gelation was re-enforced by a rheological examination of the xyloglucan systems. Fig. 1 shows the rheological properties at 37°C of gels of concentrations 0.5, 1.0 and 1.5% w/w in simulated gastric fluid, pH 1.2, from which it is clear that the 0.5% w/w systems showed very low gel strength. Values of gel strength (kN m^{-2}) at 37°C calculated from these data were 0.479, 2.377 and 2.876 for gel concentrations of 0.5, 1.0 and 1.5% w/w, respectively. The values obtained by this method are relative rather than absolute values, but serve to show the influence of gel concentration on gel strength. The observed increase of gel strength with concentration might be expected for gels of the enzyme-degraded xyloglucan, which, as shown by small-angle X-ray scattering studies (Yuguchi et al., 1997), form by the lateral stacking of rigid chains; an increase of concentration leading to an increased density of the stacked chains. The viscosity behaviour shown in Fig. 1 is typical of elastic gels, the sudden decrease of stress after the maximum indicating a brittle system.

3.2. In vitro release of indomethacin and diltiazem

The release profiles of indomethacin from xyloglucan gels loaded with 0.1% w/v drug are compared in Fig. 2 with that from a suspension of indomethacin of the same concentration. The receptor solution was changed after 1 h from a solution of pH 1.2 to one at pH 6.8 to simulate gastro-intestinal transit. No significant release of indomethacin was noted under highly acidic conditions, presumably because of the low solubility of the non-ionised 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 the gels. At pH 6.8,

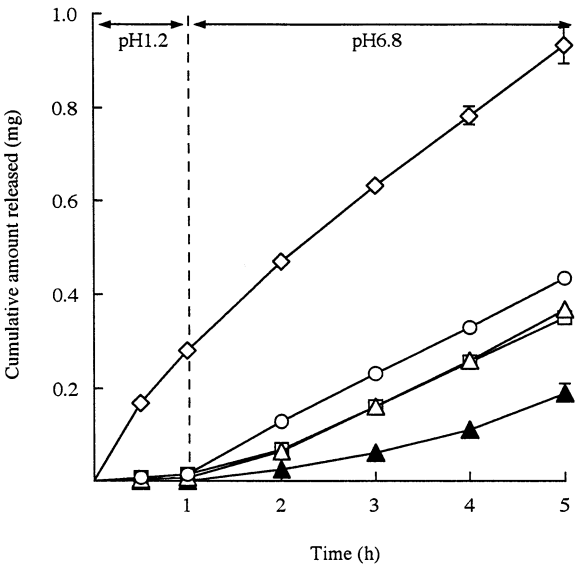


Fig. 2. Cumulative release of indomethacin as a function of time from xyloglucan gels of concentrations (○) 0.5, (△) 1.0, (□) 1.5% w/w, and from (▲) a suspension in 0.57% w/v sodium alginate and of (◇) diltiazem from 1.5% w/w xyloglucan gels. Release was into simulated gastric fluid, pH 1.2, for a period of 1 h and subsequently into simulated intestinal fluid, pH 6.8. Each value is the mean ± SE of three determinations.

indomethacin is fully ionised and will have a greater tendency to reside in the water channels of the gels from which it will be released by diffusion in the concentration gradient between donor and receptor compartments. The higher release from the 0.5% w/w xyloglucan system reflects the lower diffusional resistance of these soft gels compared with that of the rigid gels formed by 1.0 and 1.5% w/w solutions at 37°C.

Fig. 2 also shows the release profile of diltiazem from xyloglucan gels loaded with 0.1% w/v drug under identical conditions. Diltiazem (pK_a 7.7) is fully ionised at pH 1.2 and, unlike indomethacin, will partition predominantly into the water channels of the gel from which it will be readily released. Change in pH to 6.8, although reducing the percent ionisation to 88.8%, produced no significant discontinuity in the release profile.

The release data were analysed according to the treatment proposed by Higuchi (1962) for drug release from semisolid vehicles containing dissolved drug. For the initial 50–60% release, the cumulative amount Q of drug released per unit surface area is proportional to the square root of time t:

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

Indomethacin data were analysed at pH 6.8 only (where the drug is fully ionised); analysis of diltiazem data was conducted over the total pH range. Plots of Q vs $t^{1/2}$ for indomethacin in 1.0 and 1.5% w/w gels were linear after a short lag period (Fig. 3), indicating that drug release was controlled by diffusion of drug through the gel matrix. Release from the suspension and from the 0.5% w/w xyloglucan gel did not conform to eqn 1 as might be expected. Diffusion coefficients calculated from the gradients of these plots were 4.09×10^{-6} and 3.60×10^{-6} cm^2 s^{-1} for the 1.0 and 1.5% w/w xyloglucan gels, respectively. The diffusion coefficient for release of diltiazem was 7.58×10^{-6} cm^2 s^{-1}; the higher value reflecting a greater hydrophilicity of this drug compared with indomethacin.

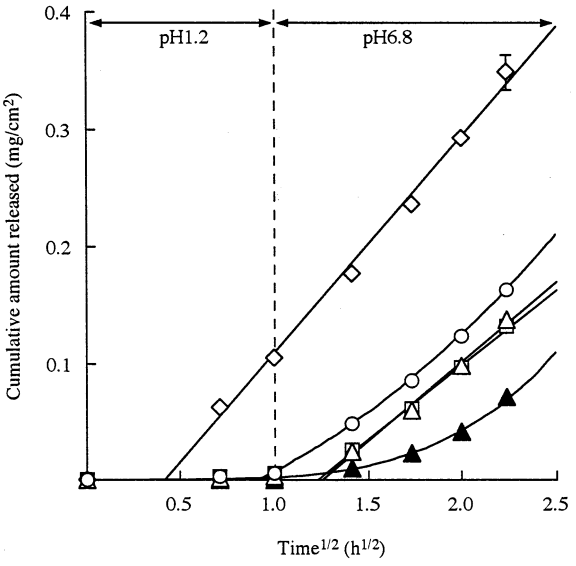


Fig. 3. Cumulative release of indomethacin per unit area, Q, as a function of root time from xyloglucan gels of concentrations (○) 0.5, (△) 1.0 and (□) 1.5% w/w, and from (▲) a suspension in 0.57%w/v sodium alginate; and of (◇) diltiazem from 1.5% w/w xyloglucan gels. Release was into simulated gastric fluid, pH 1.2, for a period of 1 h and subsequently into simulated intestinal fluid, pH 6.8. Each value is the mean ± SE of three determinations.

3.3. In vivo release of indomethacin

Plasma drug levels following oral administration to rats of 1 mg indomethacin from a chilled 1.0% w/v solution of xyloglucan (which gels in the stomach at 37°C), and from a suspension in 0.57% w/v sodium alginate are compared in Fig. 4 with those resulting from i.v. injection of an aqueous solution. Plasma levels reached a constant level of approximately 10 μg ml^{-1} 2 h after administration of the gel formulation compared with only approximately 3 μg ml^{-1} 30 min after administration of the suspension. For both of these systems, the constant levels were maintained over the period of observation (7 h). Visual observation of the contents of the stomach following administration of a 1.5% w/w xyloglucan gel (without drug) containing a marker dye showed the presence of a soft gel 7 h after dosing. The maintenance of the integrity of the gel over this time period is probably the cause of the prolongation of the release of indomethacin from the gel.

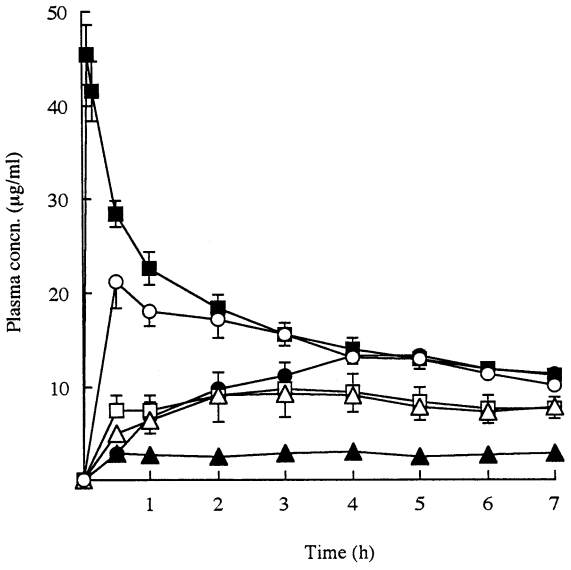


Fig. 4. Plasma concentration of indomethacin in rats following administration by (■) i.v. injection of aqueous solution, and by oral administration of (○) 0.5, (△) 1.0 and (□) 1.5% w/w xyloglucan sols, (●) 25% w/w Pluronic F127 sols, and (▲) suspension. Each value is the mean ± SE of five determinations.

The area under the plasma concentration–time curve (AUC) and the mean residence time (MRT) were obtained from the plasma concentration–time data of each animal using a personal computer program for model-independent analysis (Yamaoka et al., 1981) and are summarised in Table 1. The bioavailability of indomethacin, calculated from the ratio AUC [(oral/i.v.) × 100], was 44.7% for the xyloglucan gels, compared with only 15.7% for the oral suspension. The mean $t_{1/2}$ value was 7.24 h, which compares with a value of 5.78 h reported by Liversidge et al. (1989) for indomethacin after i.v. administration to rats. Also included in this figure are the plasma concentration profiles following indomethacin release from a 25% w/w Pluronic F127 gel, formed in situ by the administration of a chilled solution of this poloxamer containing an identical indomethacin concentration. Although peak plasma levels and bioavailabilities achieved from these concentrated poloxamer gels were higher ($C_{max} = 14.3$ μg ml^{-1}; bioavailability, 59.8%) than those from xyloglucan gels, it should be noted that these gels were of a much higher concentration than those of xyloglucan, which may introduce potential toxicity problems. It is interesting to note a divergence of the plasma concentration profiles of indomethacin from xyloglucan and F127 gels, the higher plasma levels from the F127 gel possibly being a consequence of the loosening of the structure of the gel due to its dissolution in the acid environment of the stomach after 3 h.

The influence of xyloglucan concentration on in vivo release of indomethacin (Fig. 4) was similar to that observed for in vitro release, with similar release profiles for the rigid gels formed by 1.0 and 1.5% w/w solutions. Although a higher

Table 1
Comparison of bioavailability parameters of indomethacin administered from xyloglucan gels, 25% w/w Pluronic F127 gels and suspensions[a]

	t_{max} (h)	C_{max} (μg ml^{-1})	AUC (μg h ml^{-1})[b]	AUCoral/AUC$_{i.v}$	MRT (h)[c]
I.v. injection	–	–	120.07 ± 8.58	–	2.84 ± 0.03
Xyloglucan gel					
0.5% w/w	0.80 ± 0.30	21.31 ± 2.78*	99.53 ± 6.48*	0.829 ± 0.05	3.24 ± 0.08
1.0% w/w	3.80 ± 0.92	10.82 ± 2.28**	53.71 ± 10.55**	0.447 ± 0.09	3.72 ± 0.07
1.5% w/w	3.00 ± 0.45	10.63 ± 2.56**	57.82 ± 12.26**	0.482 ± 0.10	3.64 ± 0.08
F127 gel	4.60 ± 0.51**	14.30 ± 0.60*	71.83 ± 4.34*	0.598 ± 0.04	3.98 ± 0.20
Suspension	2.20 ± 0.72	3.76 ± 0.72	18.79 ± 4.94	0.157 ± 0.04	3.56 ± 0.17

[a] Each value represents the mean ± S.E. of five experiments.
[b] Calculated from 0 to 7 h.
[c] Mean residence time.
* $P < 0.001$.
** $P < 0.05$.

bioavailability was achieved with the 0.5% w/w system (82.9%), the release was more rapid and did not show the sustained release effect noted with the rigid gel vehicles.

The reproducibility of the in vivo release data from this limited study is reasonable when it is considered that the gel masses formed in the stomach following oral administration in each of the five experiments were unlikely to have been of regular shape and hence would have been of variable surface area. Our results suggest that, at least in rat stomach, any variability of size and shape of the release depot was not sufficiently great to have a significant influence on the drug release profile.

4. Conclusion

Xyloglucan gels have potential as vehicles for oral delivery. This study has demonstrated that they may be formed in situ (in rats) by oral administration of a chilled solution, and that release of the model drug indomethacin is sustained over a time period of at least 7 h. Gelation of xyloglucan sols is not instantaneous (gelation of 1% w/v sol at 30°C occurs in 6 min (Miyazaki et al., 1998)), and it is considered that oral administration in humans may be possible, with gelation occurring in the stomach rather than in the mouth or oesophagus, although we have no evidence to support this. In addition, xyloglucan is non-toxic and has an advantage over other in situ gelling agents such as the poloxamers, of gelation at much lower concentration.

Acknowledgements

This study was supported by the Uehara Memorial Foundation Tokyo, the Japan Society for the Promotion of Science (JSPS) and the Royal Society of Great Britain. The authors are grateful to F. Suisha of the Health Science University of Hokkaido and M. Shirakawa and K. Yamatoya, Dainippon Pharmaceutical Co. for their helpful advice.

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