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RESEARCH PAPER

In Situ Gelling Pectin Formulations for Oral Sustained Delivery of Paracetamol

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ABSTRACT

The purpose of this study was to evaluate the potential of a pectin formulation with in situ gelling properties for the oral sustained delivery of paracetamol (acetaminophen). The formulations consisted of dilute aqueous solutions (1% to 2% w/v) of low methoxy pectin containing calcium ions in complexed form, which on release in the acidic environment of the stomach caused gelation of the pectin. In vitro studies demonstrated diffusion-controlled release of paracetamol from the gels over a period of 6 h. A bioavailability of approximately 96% of that of a paracetamol solution could be achieved from gels containing an identical dose of drug formed in situ in the stomachs of rats, with appreciably lower peak plasma levels and a sustained release of drug over a period of at least 6 h.

Key Words: Pectin gels; In situ gelation; Oral drug delivery; Sustained release; Paracetamol.

INTRODUCTION

Paracetamol (acetaminophen) is usually administered orally in tablet and liquid form, following which its absorption is rapid, predominantly from the small intestine. A more sustained delivery of this drug has been reported by Endo et al.[1] from an orally administered, heat-sensitive melting gel containing κ-carrageenan and gelatin as gelling agents, which achieved a high (90%) bioavailability in rabbits. We recently described an in situ gelling formulation of the polysaccharide xyloglucan[2] suitable for oral administration, from which paracetamol release (in rabbit) was sustained over a period of 5 hours, and the

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bioavailability was similar to that of a commercially available suspension (3 Months Plus Pain Relief Suspension, The Boots Co., UK). This formulation was designed to be administered in liquid form (as a chilled solution) and gelled in the stomach on attainment of body temperature. The potential of in situ gelling formulations of gellan and sodium alginate for oral sustained delivery of paracetamol has also been assessed.[3] Reproducible gelation of dilute aqueous solutions of these polysaccharides was ensured by including a source of Ca^{++} ions in the formulation, but gelation was delayed until the orally administered solution reached the stomach by complexing the calcium with sodium citrate. Here the acidic environment causes breakdown of the complex, releasing free Ca^{++} ions and causing instantaneous gelation. Similar formulations of these two polysaccharides for the oral delivery of theophylline[4,5] and cimetine[6] have been described.

In the present study we have explored the possibility of applying a similar mechanism for the in situ gelation of pectin. Pectins are a family of polysaccharides in which the polymer backbone mainly comprises α-(1→4)-D-galacturonic acid residues. Low methoxy pectins (degree of esterification <50%) such as those used in the present study readily form gels in aqueous solution in the presence of free calcium ions, which cross-link the galacturonic acid chains in a manner described by the ''egg-box'' model.[7] We investigate here the optimum quantities of complexing agents to ensure that the formulation is maintained in liquid form on oral administration (for ease of swallowing) and forms a gel suitable for the sustained release of paracetamol in the stomach. Both in vitro and in vivo release characteristics of this drug have been examined.

MATERIALS

Pectin (LM-104AS, DE=31%, Lot 23001–7) was supplied by SANSHO Co. Ltd., Osaka, Japan. Paracetamol (acetaminophen) was obtained from Yamanouchi Pharmaceutical Co., Tokyo, Japan. All other reagents were of analytical grade.

METHODS

Preparation of Sols

Pectin solutions of concentrations 1.0%, 1.5%, and 2.0% w/v were prepared by adding the pectin to ultrapure water containing 0.5% w/v (19.37 mmol L^{-1}) sodium citrate and 0.1% w/v (9.01 mmol L^{-1}) calcium chloride and heating to 40–50°C while stirring. Appropriate amounts of paracetamol (1.0% w/v) were then dissolved in the resulting solution. A 1% w/v solution of paracetamol was also prepared in ultrapure water. Assay of pectin formulations by high-performance liquid chromatography (HPLC) showed no evidence of the paracetamol degradation product p-aminophenol over the time period of the study.

Measurement of Rheological Properties of Sols and Gels

The viscosity of sols (drug-free) prepared in water was determined at 20°C with a cone and plate viscometer with cone angle 1°34′ (TV-20H, model E, Tokimec Co., Tokyo, Japan) using a 1-mL aliquot of the sample. Measurements on each sample were performed in triplicate, each taking approximately 30 s.

Gel strengths of pectin were determined at 37°C using a rheometer (CR-200D, Sun Scientific Co.,Tokyo, Japan) by the method described previously.[8] Cylindrical gels of 1–2% w/v pectin were prepared by placing a 30-mL sample of the solution into a cellulose tube (Viskase Sales Co., Chicago, IL, size 36/32), immersing the tube in 150 mL of pH 1.2 simulated gastric fluid (as specified for the JP XIV disintegration test), and allowing to equilibrate for 24 h. 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 changes in the load on the probe were measured as a function of the depth of immersion of the probe below the gel surface.

Measurement of In Vitro Drug Release

The release rates of paracetamol were measured using a plastic dialysis cell similar to that described previously.[9] The capacity of each half-cell was 4 mL and the surface area of the membranes (molecular weight cut-off 14,000) was 2.67 cm^2. Sols of pectin loaded with 1.0% w/v of drug were placed in the donor compartment. An equal volume of simulated gastric (pH 1.2) or intestinal (pH 6.8) fluid (as specified for the JP XIV disintegration test) was placed in the receptor compartment. The donor phase and the aqueous receptor phase were separated by a cellulose membrane (Viskase Sales Co., Chicago, IL, size 36/32). The assembled cell was shaken horizontally at the rate of 60 strokes min^{-1} in an incubator. The total volume of the receptor solution was removed at intervals and replaced by fresh release medium. The

drug concentration of the samples was determined using a spectrophotometer at a wavelength of 244 nm.

Animal Experiments

Male Wistar rats, weighing 250–350 g, were fasted for 24 h with free access to water. The sol preparation (1 mL) containing 10 mg paracetamol was orally administered to the anaesthetized rats (i.p., injection of urethane) using a stomach sonde needle for rats (Natume Seisakusho, KN-349D). A stomach sonde needle was also used for oral administration of the solution (10 mg in 1 mL). 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.

Determination of Paracetamol

The plasma samples were separated by centrifugation and assayed by high-performance liquid chromatography (HPLC) (Shimazu LC-10A with a Shimazu SPD-10A detector at a wavelength of 254 nm). The assay of paracetamol was based on the methods described by Ameer et al.[10] with minor modifications. To 200 μL of plasma was added 200 μL of water, 100 μL of 2-acetoaminophenol solution (100 μg mL^{-1} in 20% methanol) as internal standard, and 7 mL of ethyl acetate. The sample was vortex mixed and centrifuged, after which 5 mL of the organic layer was evaporated to dryness under a nitrogen stream. The residue was reconstituted with 200 μL of 50% methanol, and aliquots of 20 μL were injected onto a 150 × 4.6 mm i.d. column, packed with Inertsil–ODS. Elution was carried out with acetonitrile (pH 4.0):sodium acetate buffer (15:85) at a rate of 0.8 mL min^{-1} at 40°C.

RESULTS AND DISCUSSION

Gelling Properties

Although gelation of polysaccharides such as alginate and pectin will occur in the presence of H^+ ions, a source of divalent ions, generally Ca^{++}, is usually required to produce gels that are suitable as vehicles for drug delivery. In this study, Ca^{++} ions in complexed form were included in the formulation for induction of pectin gelation in the stomach. Sufficient sodium citrate was added to the pectin solution to form a complex with most of the Ca^{++} ions present and hence to effectively remove them from solution. By this means the formulation was maintained in a fluid (sol) state until the breakdown of the complex in the acidic environment of the stomach, release of Ca^{++} ions then causing gelation to occur. The optimum quantities of calcium chloride and sodium citrate that maintained fluidity of the formulation before administration and resulted in gelation when the formulation was added to simulated gastric fluid, were determined by preliminary tests in which pectin sols (1.5% w/v) containing sodium citrate concentrations in the range 0–0.75% w/v and calcium chloride concentrations of 0.05%, 0.075%, and 0.10% w/v were added dropwise to 50 mL simulated gastric fluid (pH 1.2, 37°C). Of the sodium citrate concentrations examined, the minimum concentration required to maintain the formulations in the sol state and ensure their gelation in acidic conditions with each of these three calcium chloride concentrations was 0.50% w/v. Gelation occurred without exposure to acidic conditions in formulations containing sodium citrate concentrations of 0.25% w/v or less and either 0.075% or 0.10% w/v $CaCl_2$; these formulations were not therefore of interest for the

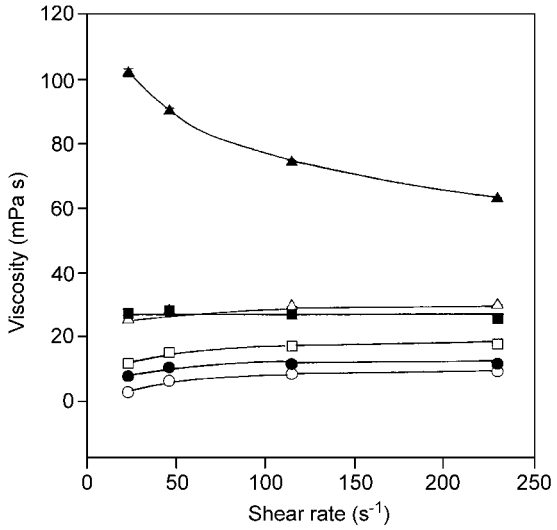

Figure 1. Influence of the calcium citrate complex on the viscosity of pectin sols of concentrations (○) 1.0, (□) 1.5, and (△) 2.0% w/v at 20°C; closed symbols refer to pectin sols containing 0.1% $CaCl_2$ and 0.5% sodium citrate, open symbols refer to sols with no added ions. Each value is the mean ± S.E. of three determinations; error bars are within the size of the symbol.

present study. The optimum formulation for maximum gel strength was 0.10% w/v calcium chloride and 0.50% w/v sodium citrate; further increase of calcium chloride content with the same sodium citrate concentration caused gelation of the formulation before contact with simulated gastric fluid.

The influence of the calcium citrate complex on the viscosity of pectin sols in water at 20°C is shown in Fig. 1. Pectin sols not containing added complexing agents showed Newtonian flow properties, the viscosity of the sols increasing with the pectin concentration as expected. Addition of calcium chloride and sodium citrate increased the viscosity, particularly of 2% w/v pectin sols where shear thinning flow properties were observed. However, the viscosities of all of these formulations are sufficiently low that no difficulties with swallowing are envisaged. The observed increase of viscosity is most likely to be a consequence of weak gelation caused by the cross-linking of the pectin chains by free Ca^{++} ions in the solutions that have not been complexed by the citrate anion. There will also be a small increase of ionization of the pectin (pK_a of $COO^- = 3.5$) arising from an increase of pH of solution when the complex is added (pH 5 increased to 6), which could affect the interaction between the chains, although this effect is likely to be small.

Figure 2 compares the rheological properties of gels formed in the absence and presence of Ca^{++} ions. Gel behavior under applied stress was determined using a simple method that measured the change in load of a probe pushed slowly through the gel. Gels formed in the presence of Ca^{++} ions showed behavior typical of elastic gels, the sudden decrease of stress after the maximum indicating a brittle system. This type of gel has been noted previously with other polysaccharide gels, including xyloglucan[11] and gellan.[3] Values of the stress immediately before collapse, which may be used as a guide to gel strength, for 1.0%, 1.5%, and 2.0% w/v pectin gels were 14.3, 33.9, and 44.7 kN/m^2, respectively. These values are considerably lower than gel strengths measured using the same apparatus for other polysaccharide gels, for example 1% gellan (190.8 kN/m^2) and 1.5% alginate (79.9 kN/m^2).[3] Nevertheless, our observations (see below) have shown that the pectin gels have sufficient strength to maintain their integrity for several hours in the stomach. Similar rheograms were obtained with 1.5% and 2.0% w/v pectin formulations not containing Ca^{++} ions. These gels, which are cross-linked by H^+ ions, had similar gel strengths to those in which chains were linked by Ca^{++} ions but collapsed at appreciably lower strains. It is interesting to note that 1.0% w/v pectin gels in which chains are cross-linked by Ca^{++} ions show properties characteristic of elastic gels, whereas those formed in the absence of these ions were soft gels not suitable for use as vehicles for drug delivery.

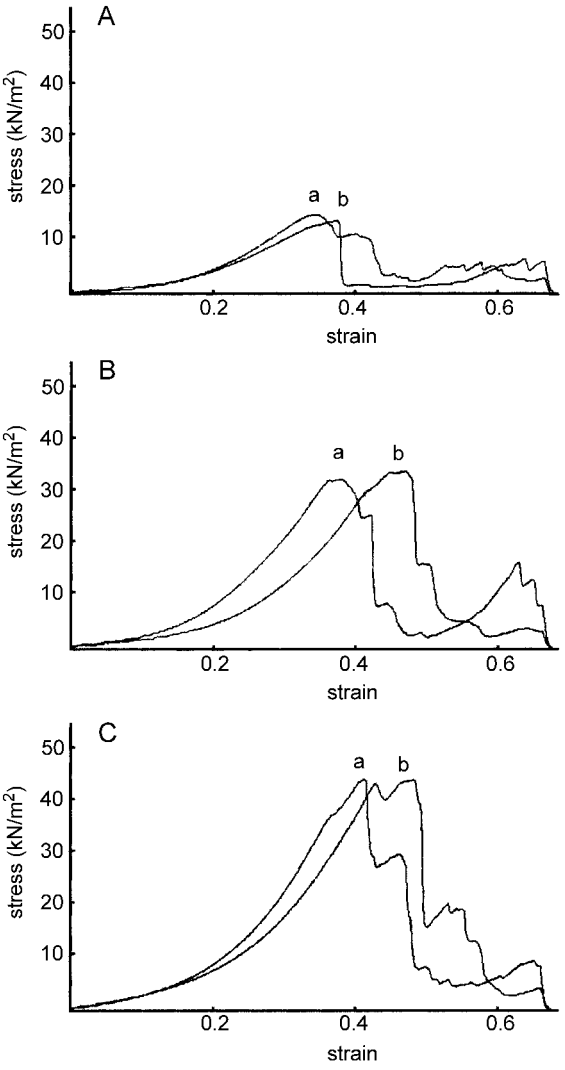

Figure 2. Influence of the calcium citrate complex on the rheological properties of (A) 1.0%, (B) 1.5% and (C) 2.0 % w/v pectin gels in simulated gastric fluid at pH 1.2 and 20°C; (a) with no added ions and (b) containing 0.1% $CaCl_2$ and 0.5% sodium citrate.

In Vitro Drug Release

The release profiles of paracetamol from 1.0% w/v gels formed in the presence and absence of free Ca^{++} ions are compared with that from an aqueous solution of paracetamol of the same concentration (1% w/v) in Fig. 3. The receptor solutions 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 pK_a of paracetamol is 9.5[12] and

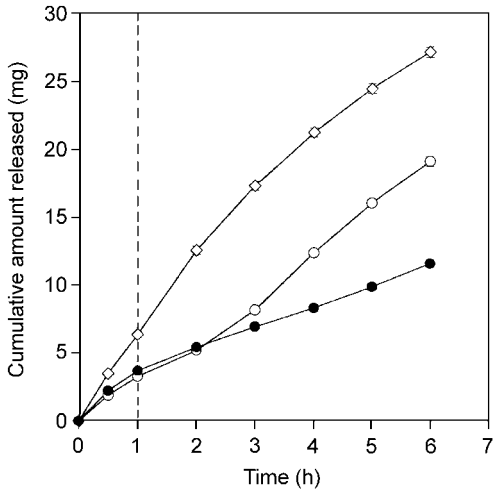

Figure 3. In vitro release of paracetamol at 37°C plotted as cumulative release against time from (○) 1.0% w/v pectin sols and (●) 1.0% w/v pectin sols containing 0.1% $CaCl_2$ and 0.5% sodium citrate, and (◇) an aqueous solution of paracetamol. All formulations initially contained 1.0% w/v paracetamol. 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 four determinations; error bars are within the size of the symbol.

consequently there will be no change in the state of ionization of this acidic drug accompanying this pH change. For gels not containing free Ca^{++}, there was a pronounced increase of release following this pH change; no such inflection was observed for gels formed in the presence of Ca^{++} ions. Observation of the contents of the donor cells during release measurements showed that the inflection in the plots for release from the calcium-free pectin gels coincided with a reversion of these gels back to the sol phase as the pH in the receptor cell was changed from pH 1.2 to pH 6.8, suggesting that these gels had insufficient strength to withstand a large decrease of hydrogen ion concentration. The cumulative amount released from these formulations approached that from the paracetamol solution, as expected for release from sol phase. In contrast, the gels formed by complexation with calcium ions retained their gel structure throughout the release experiment. Plots showing similar release profiles to those of Fig. 3 were obtained for 1.5% and 2.0% pectin gels.

The release data over the whole time period from the pectin gels formed by complexation with calcium were analyzed according to the treatment proposed by Higuchi[13] 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 from gels of initial drug concentration C_0 is proportional to the square root of time t:

$$Q = 2\,C_0\,(D\,t\,/\,\pi)^{1/2} \quad (1)$$

Plots of Q *vs.* $t^{1/2}$ for the release of paracetamol from the pectin gels are shown in Fig. 4. Release from 1.5% and 2.0% w/v gels conformed to Eq. 1 after a short lag period, indicating diffusion-controlled release. The diffusion coefficients, D, calculated from the gradients of the plots for 1.5% and 2.0% w/v gels were $5.75 \pm 0.36 \times 10^{-6}$ and $5.97 \pm 0.27 \times 10^{-6}$ $cm^2\ s^{-1}$ (n = 4± S.E.), respectively. There is evidence of departure from linearity at longer release times in the plot of release from 1% w/v gels. A diffusion coefficient of $6.59 \pm 0.31 \times 10^{-6}$ $cm^2\ s^{-1}$ (n = 4 ± S.E.) was calculated from the gradient of this plot assuming linearity during the initial 4 h of release, but clearly this value should be treated with some caution.

In Vivo Release

Plasma drug levels following oral administration to rats of paracetamol (10 mg) from 1.0%, 1.5%, and 2.0% w/v pectin sols containing the optimum levels of sodium citrate and calcium chloride, from a 1.5% w/v pectin sol without complexing agents, and from a

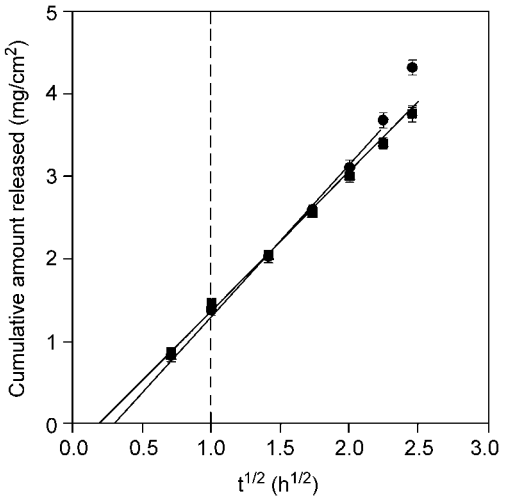

Figure 4. In vitro release of paracetamol at 37°C plotted as cumulative release against square root time from (●) 1.0%, (■) 1.5%, and (▲) 2.0% w/v pectin sols containing 0.1% $CaCl_2$ and 0.5% sodium citrate. All formulations initially contained 1.0% w/v paracetamol. Release was into simulated gastric 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 four determinations.

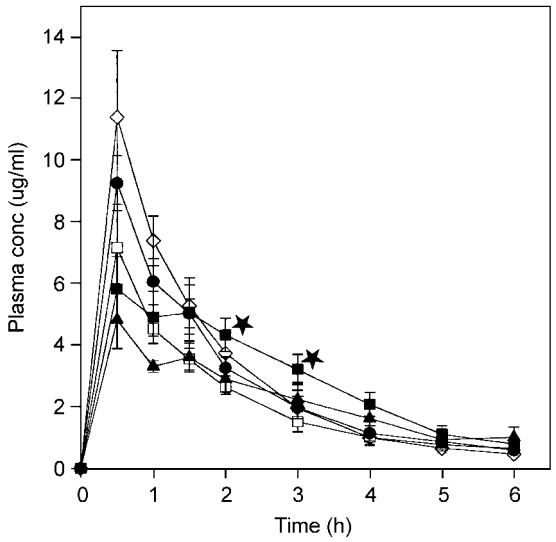

Figure 5. Plasma concentrations of paracetamol in rats after oral administration of (●) 1.0%, (■) 1.5%, and (▲) 2.0% w/v pectin sols containing 0.1% $CaCl_2$ and 0.5% sodium citrate, (□) 1.5% w/v pectin sols with no added ions, and (◇) an aqueous solution of paracetamol. All formulations initially contained 10 mg paracetamol. Each value is the mean ± S.E. of 4 determinations. *$p<0.05$ compared with 1.5% w/v pectin sols not containing complexing agents.

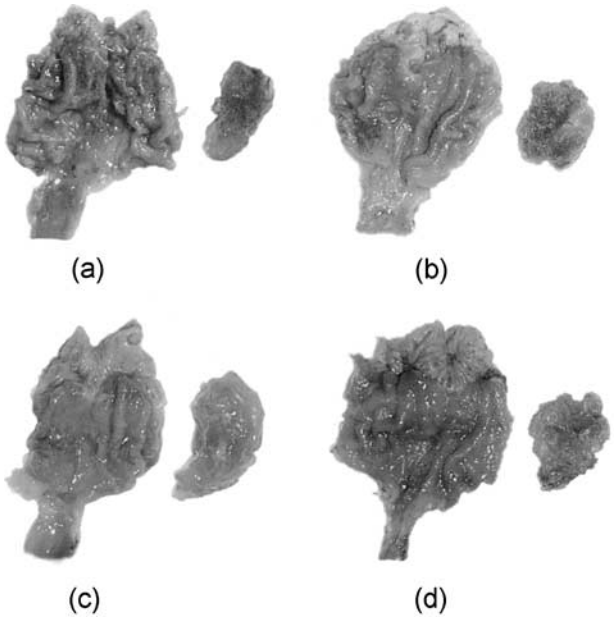

Figure 6. Photographs showing the gels formed in rat stomach 3 h after oral administration of (a) 1.0%, (b) 1.5%, and (c) 2.0% w/v pectin sols and (d) 5 h after oral administration of 1.5% w/v pectin sol.

solution of paracetamol (10 mg in 1 mL), are compared in Fig. 5. Gelation of the pectin sols was confirmed by visual observation of the stomach contents, which showed the presence of distinct gel blocks of regular shape (as discussed below). Rapid absorption from the solution produced a peak plasma drug concentration, C_{max}, of 11.4 μg mL^{-1} at a time, t_{max}=0.5 h. A more sustained release of drug from the gels was evident from the concentration-time profiles of Fig. 5; the C_{max} decreasing and the t_{max} increasing with increase of pectin concentration (see Table 1). Comparison of the profiles for 1.5% gels with and without added complexing agents shows a more sustained release from the gels formed in the presence of calcium ions, presumably because of their more structured nature and a consequent greater resistance to the diffusion of drug. The addition of complexing agents to the 1.5% w/v pectin formulations significantly ($p<0.05$) increased plasma concentrations of paracetamol at 2 and 3 h.

The area under the plasma concentration-time 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[14] are summarized in Table 1. Bioavailabilities approaching that from the paracetamol solution were

Table 1. Comparison of bioavailability parameters of paracetamol administered from aqueous solution and from pectin 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)	AUC_{gel} AUC_{soln}
1.0% w/v pectin	9.26±0.97	0.50±0.00	16.96±2.10	1.80±0.06	0.91±0.11
1.5% w/v pectin without Ca^+	7.36±1.23	0.63±0.13	13.22±0.57	1.89±0.19	0.71±0.03
1.5% w/v pectin	6.30±1.04	1.00±0.29	17.93±2.40	2.24±0.15[a]	0.96±0.13
2.0% w/v pectin	5.08±0.77[a]	1.13±0.38	13.34±1.29	2.25±0.21[a]	0.71±0.07
Paracetamol soln	11.40±2.18	0.50±0.00	18.70±2.17	1.62±0.13	1.00

Note: Each value represents the mean ± S.E. (n = 4).
[a]$p<0.05$ compared with aqueous solution.

achieved with the 1.5% w/v pectin gel with a significantly ($p<0.05$) higher mean residence time.

Visual observation of the contents of the rat stomach following administration of 1 mL of 1.5% w/v pectin sol containing a marker dye (but without drug) showed the changes in the amount of gel present over a 5-h period, with approximately 42% of the gel remaining at 5 h after administration (Fig. 6). The maintenance of the integrity of the gel in the stomach over this time period is probably the cause of the prolongation of the release of paracetamol from the gel. Similar studies comparing the amounts of gel remaining in the stomach at 3 h after administration of 1.0%, 1.5%, and 2.0% w/v sols produced values of 30.4%, 50.5%, and 82.9%, respectively (see Fig. 6), the increase in amount remaining with increase of pectin concentration reflecting the increase of gel strength with increase of pectin concentration shown in the rheological studies.

CONCLUDING REMARKS

This study has demonstrated the potential of an orally administered in situ gelling pectin formulation for the sustained delivery of paracetamol. We have shown that dilute aqueous solutions, containing pectin and a source of calcium in complexed form, gel when the calcium ions are released in the acidic environment of the rat stomach, and the gels function as depots for the release of paracetamol. Peak plasma levels of paracetamol from the in situ gelling formulations were appreciably lower than those following oral administration of a solution of this drug of the same concentration, and drug release was sustained over a period of at least 6 h. A bioavailability in rat of approximately 96% of that from the solution was achieved from a 1.5% w/v pectin gel.

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