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In situ gelling pectin formulations for oral drug delivery at high gastric pH

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Abstract

The aim of the study was to compare the gelation and drug release characteristics of formulations of pectin with high (31%) and low (9%) degrees of methoxylation over a wide pH range (pH 1.2–5.0). Dilute solutions of pectin (1.5%, w/v) containing complexed calcium ions formed gels in vitro at low pH (pH < 2.5) as a consequence of cross-linking of the galacturonic chains by calcium ions released from the complex, but the efficiency of gelation was significantly reduced with increase of pH because of incomplete release of complexed Ca^{++}. Gelation of formulations of pectin with a degree of esterification of 9% (DE9) was observed over the pH range 2.5–5.0 in the presence of 1.6 mM Ca^{++}, but was incomplete in formulations of pectin with a degree of esterification of 31% (DE31). A sustained release of ambroxol was observed following oral administration of pectin DE9 formulations to gastric-acidity controlled rabbits at pH 5.5–5.7 and visual observation of the stomach contents of these rabbits confirmed in situ gelation of these formulations. There was no evidence of in situ gelation of pectin DE31 formulations under these conditions and a rapid initial drug release was observed. Differences in gelling characteristics in this pH range were attributed to the greater susceptibility of low methoxylated pectin to cross-linking by di- and tri-valent ions present in the gastric juice. It is concluded that formulations of pectin with a low degree of esterification have potential application as in situ gelling vehicles for the sustained delivery of drugs following oral administration under conditions of high gastric pH.

Keywords: In situ gelation; Oral drug delivery; Sustained release; Pectin gels; Gastric-acidity; Ambroxol

1. Introduction

The polysaccharide pectin consists mainly of α-(1,4)-D-galacturonic acid monosaccharide units that may be methyl-esterified to varying extents. The properties of pectin are significantly influenced by the degree of esterification (DE); low methoxy pectins (DE < 50%), for example, readily form gels in aqueous solution in the presence of free Ca^{++} ions, which cross-link the galacturonic acid chains.

We have recently examined the influence of gastric pH on the gelation of dilute (1.0–2.0%, w/v) aqueous solutions of pectin (DE = 31%) and on the in vitro and in vivo release of paracetamol and ambroxol (Itoh et al., 2006) from the resultant gels. It has been reported (Moore and Makhlouf, 1968) that human gastric juice contains approximately 1.6 mM Ca^{++}. In addition to calcium there are other divalent and also trivalent ions present in the stomach that are capable of forming cross-links with pectin chains (Tho et al., 2003). The concentration of these ions is, however, variable depending on food and liquids ingested. To ensure effective gelation of the pectin solutions, a source of calcium in complexed form was included in these formulations which released calcium ions when the complex was broken down in the acidic environment of the stomach. Gels suitable as sustained release vehicles were formed in situ at low pH. However, very weak gels were formed at pH 3.0 resulting in poor sustained release characteristics compared with those at pH 1.2; no significant in vitro gelation was observed at pH 3.5 (Itoh et al., 2006).

These studies are relevant to the design of in situ gelling formulations in view of the many factors that may affect gastric pH. In healthy young Caucasians the gastric pH is less than pH 3 during 90% of the fasted state, although on a minute-to-minute

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basis may reach as high as pH 7 (Dressman et al., 1990). After ingestion of a meal the gastric-acidity can vary over a wide range depending on the composition of the meal but is typically in the range pH 3–7. There is a decrease of acid secretion with age which may result in very low gastric hydrogen ion concentrations in the elderly (Morihara et al., 2001). Other influences on gastric pH include several pathophysiological conditions and also proton pump inhibitors (Hörter and Dressman, 1997).

A decrease in the degree of methylation increases the hydrophilicity of the pectin and also its susceptibility to cross-linking by divalent cations (Fishman and Hicks, 2005). Consequently, low methoxy pectin may have potential for gelation over a wider pH range than observed with the pectin sample examined previously. In the present study, we have investigated the possibility of using pectin of very much lower degree of esterification (average DE = 9%) in the formulation of in situ gelling formulations and have examined the sustained release characteristics using ambroxol as a model drug.

2. Materials and methods

2.1. Materials

Pectin with degrees of esterification of 6–12% (pectin DE9, LM-5CSJ, Lot S34305) and 31% (pectin DE31, LM-104AS, Lot 23001-7) were supplied by SANSHO Co. Ltd., Osaka, Japan. Characterisation of the pectins by GPC gave number-average (M_n) and weight-average (M_w) molecular weights of 7.34×10^4 and 26.93×10^4, respectively, for pectin DE9, and 11.6×10^4 and 29.5×10^4, respectively, for pectin DE31. Ambroxol hydrochloride (Lot YT-13) was supplied by YIA Co. Ltd., Shiga, Japan. All other reagents were of analytical grade.

2.2. 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 appropriate amounts of sodium citrate and calcium chloride and heating to 40–50 °C while stirring. The optimum amounts of these compounds which maintained the formulation in the sol state but were sufficient to ensure gelation in acidic conditions were determined from tests in which pectin DE9 sols (1.5%, w/v) containing 0.5, 0.75 and 1.0% (w/v) sodium citrate and 0.05, 0.075 and 0.10% (w/v) calcium chloride were added dropwise to 50 ml simulated gastric fluid (pH 1.2, 37 °C). In selected experiments sufficient additional calcium chloride was added to produce a free Ca^{++} ion concentration of 1.6 mM. Ambroxol hydrochloride was added to the solution as required at a concentration of 0.6% (w/v).

2.3. Measurement of rheological properties of sols and gels

The viscosity of 1.0, 1.5 and 2.0% (w/v) formulations of pectin DE9 or DE31 (containing complexed calcium but no drug) was determined at 20 °C with a cone and plate viscometer with cone angle 1°34′ (TV-20H, model E, Tokimec Co., Tokyo) using a 1 ml aliquot of the sample. Measurements on each sol were performed in triplicate over a shear rate range of approximately 20–230 s^{-1}, each shear rate sweep taking approximately 30 s.

Gel strengths of the pectin formulations were measured at 37 °C using a rheometer (CR-500DX, Sun Scientific Co., Tokyo) by the method described previously (Watanabe et al., 1994). Cylindrical gels of 1.0, 1.5 and 2.0% (w/v) pectin DE9 or DE31were prepared by placing 30 ml of the sol into cellulose tubing (Viskase Sales Co., 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 equilibrating for 24 h. The cylindrical gels (15 mm diameter and 15 mm height), formed as a result of the release of complexed calcium ions in the acidic environment, 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.

2.4. Gelation of pectin sols

The influence of pH on the gelation characteristics of 1.5% (w/v) pectin sols (DE9 and DE31) was determined by immersion of 30 ml sol enclosed in dialysis tubing (Viskase Sales Co., Chicago, USA, size 36/32) into dilute solutions of HCl (150 ml) with pH values over the range 1.0–5.0. After equilibration for 24 h at room temperature, the contents of the tube were passed through a sieve (JP XIV, No. 6.5, 2.80 mm) over a period of 30 s and the weight of the gel remaining in the sieve was determined (electronic balance, BL-220H, Shimadzu Ltd., Kyoto, Japan). The experiments were repeated in the presence of added 1.6 mM Ca^{++}.

2.5. Measurement of in vitro drug release

The in vitro release of ambroxol from pectin formulations was measured 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. Sols of 1.5% (w/v) pectin (DE9 and DE31) loaded with 0.6% (w/v) of ambroxol hydrochloride, were placed in the donor compartment. An equal volume of simulated gastric (pH 1.2, 3.0, 5.0) or intestinal (pH 6.8) fluid (as specified for the JP XIV disintegration test) was placed in the receptor compartment. The donor and receptor compartments were separated by a cellulose membrane (Viskase Sales Co., Chicago, USA, 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 measurements were repeated with sols containing added 1.6 mM Ca^{++}. The concentration of ambroxol released as a function of time was determined by HPLC as described below.

2.6. Animal experiments

2.6.1. Gastric-acidity controlled rabbits

White male rabbits weighting 2.8–3.2 kg were used for the experiment. The pH control of the gastric contents was per-

formed by the method of Takahashi et al. (1983). Each rabbit was fed 100 g day^{-1} of special solid diet (CR-3 not containing alfalfa, Clea Japan Inc., Tokyo, Japan) for 3 days. At the end of this period, it was fasted for 24 h, and then fed 50 g day^{-1} of the special soft diet (30 g of water was added to 20 g of special solid diet) for 1 day. Finally, on the day before administration of the pectin formulation and also again 30 min before administration of the formulation, it was fed the 50 g of the special soft diet containing 15 mg of lansoprazole (Takepron® Capsules 15, Takeda Pharmaceutical Company Ltd., Osaka, Japan).

The intragastric pH of anaesthetised (pentobarbital 25 mg kg^{-1} by i.v. injection) gastric-acidity controlled rabbits was measured by a pH meter (F-22, Horiba Ltd., Tokyo, Japan) using a micro pH glass electrode (CM-181, Chemical Instruments Co., Tokyo, Japan) inserted in the stomach and a comparative ceramic electrode (CMR-535, Chemical Instruments Co.) glued to the leg.

2.6.2. In vivo drug release

A 1.5% (w/v) pectin sol preparation containing 24 mg/4 ml ambroxol hydrochloride was administered orally using a stomach sonde needle for rabbits (KN-342 Natume Seisakusho Co., Ltd., Tokyo, Japan). Blood samples were taken from the ear vein at predetermined intervals 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 the results are presented as the mean ± standard error.

2.7. Ambroxol assay

The plasma samples were separated by centrifugation and their ambroxol content was determined by the HPLC method described by Botterblom et al. (1987) with minor modifications. A Shimadzu LC-10A HPLC with a Shimadzu SPD-10A detector was used at a wavelength of 210 nm. To 0.5 ml of plasma was added 100 μl of propranolol hydrochloride solution (0.2 μg ml^{-1}) as internal standard, 100 μl of 1 M NaOH and 5 ml of diethyl ether. The sample was vortex-mixed and centrifuged and 150 μl of 0.01 M HCl was added to the supernatant. After shaking and centrifugation, the diethyl ether layer was discarded and 50 μl of the acid layer were injected onto the analytical column (300 mm × 3.9 mm i.d.), packed with Waters μBondapak C18. A column (20 mm × 3.9 mm i.d.) packed with Waters μBondapak C18 was used as a guard column. Elution was carried out with acetonitrile–methanol–0.05 M phosphate buffer (0.65:1:3) at a rate of 0.8 ml min^{-1} at 40 °C.

3. Results and discussion

3.1. Gelation characteristics of pectin sols

3.1.1. Formulation of pectin DE9 sols

The formulations of this study contained Ca^{++} ions in complexed form, the release of which in the acidic conditions of the stomach ensured reproducible gelation of the pectin. The quantities of the complexing agents calcium chloride and sodium citrate must be such that there is no free calcium in ionic form in the formulation so ensuring that they are fluid before administration, but sufficient Ca^{++} ions are released when the complex is broken down in the stomach to cause gelation. Determination of the optimum amounts of these compounds for pectin DE9 sols (1.5%, w/v) (see Section 2.2) showed that only those containing 0.05% (w/v) calcium chloride in combination with either 0.75% (w/v) or 1.0% (w/v) sodium citrate were satisfactory; all other formulations gelled before contact with gastric fluid. All further experiments in this study were performed with formulations containing 0.05% (w/v) (4.50 mM) calcium chloride and 0.75% (w/v) (29.06 mM) sodium citrate. In comparative experiments using DE31 pectin the optimum quantities of complexing agents used were 0.10% (w/v) (9.01 mM) calcium chloride and 0.50% (w/v) (19.37 mM) sodium citrate (Kubo et al., 2004).

3.1.2. Rheological properties of pectin gels

The results of a comparative study of the viscosity of pectin formulations prepared with DE9 and DE31 pectin is shown in Fig. 1. All sols prepared with pectin DE9 were Newtonian, whereas the 2.0% (w/v) pectin DE31 formulation showed shear thinning properties. Pectin DE9 formulations had lower viscosities than those of equivalent concentrations prepared with pectin DE31, for example a 1.5% (w/v) pectin sol prepared with DE9 pectin had the same viscosity as a 1.0% (w/v) sol formulated with DE31. This difference in behaviour is an expected consequence of the higher molecular weight of the DE31 pectin. The viscosities of the formulations, particularly those containing pectin DE9, are sufficiently low that no difficulties with swallowing are envisaged.

Fig. 2 compares the rheological properties of the two formulations. Gel behaviour under applied stress was determined using a simple method that measured the change in load of a probe pushed slowly through the gel. Rheograms of both were typical

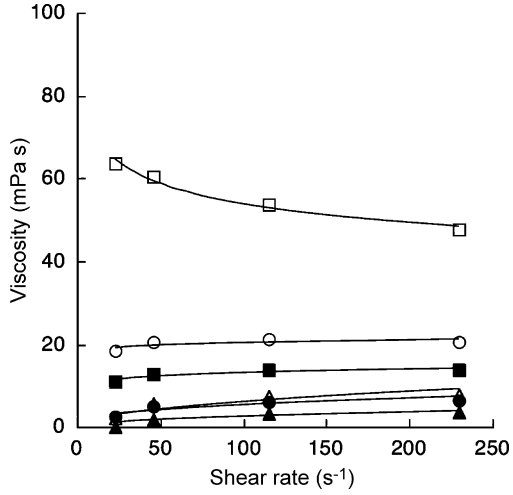

Fig. 1. Comparison of the viscosities of pectin solutions of concentrations (%, w/v): 1.0 (triangles), 1.5 (circles) and 2.0 (squares) at 20 °C. Closed symbols refer to solutions formulated with pectin DE9, open symbols refer to pectin DE31. Each value is the mean ± S.E. of three determinations.

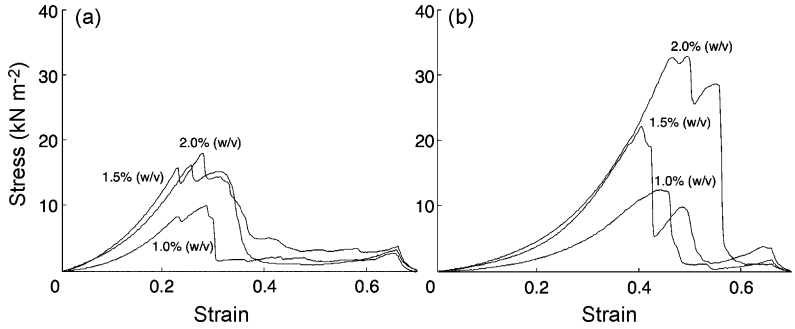

Fig. 2. Rheological properties at 37 °C of pectin solutions with concentrations indicated, formulated with (a) pectin DE9 and (b) pectin DE31, in simulated gastric fluid pH 1.2.

of elastic gels, the abrupt decrease of stress at high strain indicating a brittle system. 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 DE31 gels were 13.29, 23.46 and 34.68 kN m^{-2}, respectively; those for the pectin DE9 gels were 9.50, 17.17 and 19.03 kN m^{-2}, respectively, indicating that much softer gels are formed with the shorter chains of pectin DE9.

3.1.3. Effect of pH and added Ca^{++} ions on gelation

It has been estimated that there is about 1.6 mM of non-parietal (non-secreted) calcium present in the normal human gastric interstitial fluid (Moore and Makhlouf, 1968). The effect of this calcium, which is in addition to the ionized calcium released from the calcium complex in the stomach, on the gelation of formulations prepared with both types of pectin is compared in Fig. 3. The results are presented as the weight of gel formed from 30 ml solutions containing 1.5% (w/v) pectin in the presence and absence of 1.6 mM Ca^{++}, after dialysis in solutions of HCl over the pH range 1–5. The results show that the hydrogen ion concentration at pH 1.0–2.0 (DE9) and 1.0–2.5 (DE31) was sufficiently high to release the complexed calcium ions and cause satisfactory gelation in the absence of an additional source of calcium. Visual observation showed well-defined compact gels over this pH range. There were, however, insufficient H^+ ions at higher pH to cause effective breakdown of the calcium complex in the pectin solutions and gelation was poor or non-existent (pH > 3.5) under these conditions. Fig. 3a shows that the addition of 1.6 mM Ca^{++} ions was sufficient to cause almost complete gelation of formulations containing pectin DE9 over the entire pH range examined. In contrast, although gelation of pectin DE31 formulations was observed at pH 3.0–5.0, the amount of gel formed was appreciably less (Fig. 3b). The gels formed by both formulations at pH > 2 had a looser, less structured appearance (Fig. 4).

3.2. In vitro drug release

3.2.1. The effect of pH

Fig. 5a shows similar in vitro release characteristics of ambroxol from the 1.5% (w/v) pectin DE9 and pectin DE31 formulations when 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. Visual observations of the diffusion cell showed effective gelation under these conditions. The release data over the whole time period of release for these formulations were analysed using the Higuchi equation for drug release from semisolid vehicles containing dissolved drug (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 the initial drug concentration, and t is the time.

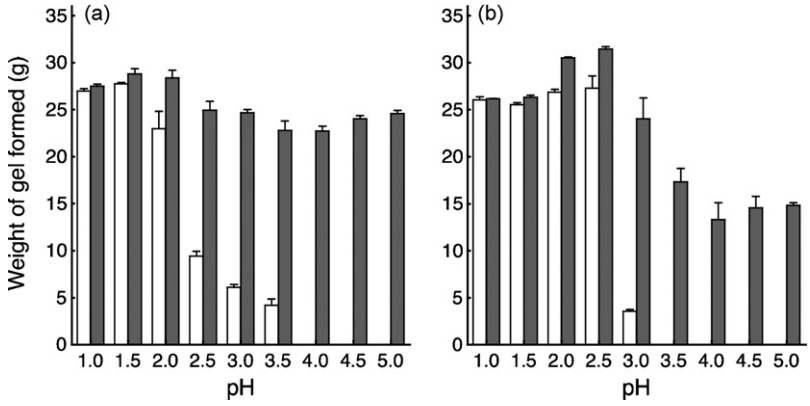

Fig. 3. The effect of added 1.6 mM Ca^{++} on the weight of gel formed from 30 ml solutions (1.5%, w/v) of (a) pectin DE9 and (b) pectin DE31, as a function of pH. Shaded columns refer to solutions containing added 1.6 mM Ca^{++}. Each value is the mean ± S.E. of 3–5 determinations.

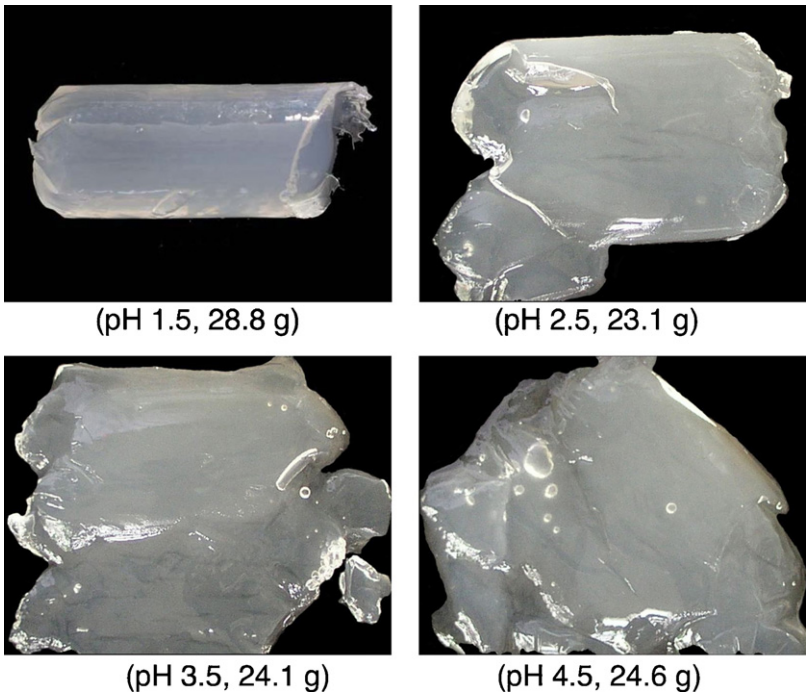

Fig. 4. Photographs showing the appearance of gels formed at pH 1.5, 2.5, 3.5 and 4.5 from 1.5% (w/v) pectin DE9 formulations in the presence of added 1.6 mM Ca^{++}. Numbers in parenthesis indicate pH and weight of the gel formed.

The values of the diffusion coefficients, D, ($\times 10^{-6}\,cm^2\,s^{-1}$) for formulations prepared with pectin DE9 and pectin DE31 determined from the gradient of the linear plots of Q versus $t^{1/2}$ were 3.61 ± 0.42 and 3.62 ± 0.60, respectively.

In both formulations release of drug was appreciably faster when the pectin was exposed to receptor solutions at pH 3.0 over the initial 1 h release period. Observation of the donor cells showed that the formulations were predominantly in sol form throughout the duration of the release period. Diffusion through the dialysis membrane of H^+ ions from the receptor solution at this pH was insufficient to cause the release of complexed calcium ions and consequently gelation of the pectin was incomplete. No further analysis of the data for these formulations was undertaken.

3.2.2. *The effect of added Ca^{++}*

The influence of added 1.6 mM Ca^{++} on drug release from pectin DE9 and DE31 formulations exposed to receptor solutions at pH 3.0 is compared in Fig. 5b. In these experiments the pH was maintained at this value throughout the duration of the measurements. Observations of the donor cells during release experiments for both formulations showed the presence of a thin gel layer on the surface of the cellulose membranes when calcium was included in the formulation but no gelation of the bulk of the sol. No attempts were made to calculate diffusion coefficients for these formulations because of their incomplete gelation, but the plots of Fig. 5b indicate a tendency for a slower diffusion rate in the pectin DE9 formulation containing added Ca^{++} ions reflecting the thicker surface gel layer

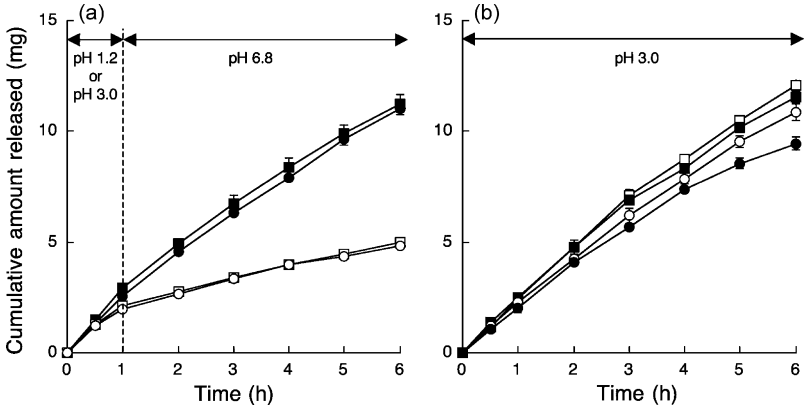

Fig. 5. In vitro release of ambroxol from solutions (1.5%, w/v) of pectin DE9 (circles) and pectin DE31 (squares) plotted as cumulative release against time. (a) Release was into simulated gastric fluid pH 1.2 (open symbols) or pH 3.0 (closed symbols) for a period of 1 h and subsequently into simulated intestinal fluid pH 6.8. (b) Release at pH 3.0 from formulations without added Ca^{++} (open symbols) and formulations containing 1.6 mM Ca^{++} (closed symbols). Each value is the mean ± S.E. of four determinations.

Table 1
Comparison of pharmacokinetic parameters of ambroxol from 1.5% (w/v) pectin gels of different degrees of esterification (DE) formed in situ in gastric-acidity controlled rabbit stomach

Pectin	Gastric pH	C_{max} (ng ml^{-1})	t_{max} (h)	AUC (0–12 h) (ng h ml^{-1})	MRT (h)
DE9	5.53 ± 0.19	78.05 ± 19.09	1.83 ± 1.09	396.21 ± 67.86	4.23 ± 0.56
DE31	5.70 ± 0.08	177.25 ± 37.69	0.50 ± 0.00	492.44 ± 174.22	3.22 ± 0.22

Each value represents the mean ± S.E. of three experiments.

in this formulation. Similar release plots were obtained at pH 5.0 (not shown) but observation of the donor compartments showed the presence of a surface gel layer only in formulations prepared using pectin DE9 in the presence of added calcium.

3.3. In vivo drug release

The pharmacokinetics of ambroxol when released from in situ gelling pectin formulations prepared using pectin DE9 and DE31 were determined after oral administration to gastric-acidity controlled rabbits at high gastric pH. The mean value of intragastric pH just before administration of pectin formulations was 5.62 ± 0.10 ($n = 6 \pm$ S.E.). Plasma drug levels following oral administration of ambroxol (24 mg/4 ml) from 1.5% (w/v) pectin sols formulated with pectin DE9 and DE31 are compared in Fig. 6. The areas under the plasma concentration–time curve (AUC) and the mean residence time (MRT) calculated from the plasma concentration–time data of each animal using a computer program for non-compartment, model-independent analysis (Yamaoka et al., 1981) are compared in Table 1.

Rapid absorption from pectin DE31 formulations produced a peak plasma drug concentration of 177 ng ml^{-1} at 0.5 h. This is in marked contrast to absorption from the pectin DE9 formulations which was maintained at approximately 50–80 ng ml^{-1} for the first 4 h after administration. Similar plasma drug concentrations were observed from both formulations after 3 h with a slow decrease in both to approximately 14 ng ml^{-1} at 8 h. Table 1 shows a longer mean residence time but a lower AUC following release from pectin DE9 formulations. Photographs of the contents of rabbit stomachs at time intervals of 1 and 5 h after administration of 4 ml of pectin DE9 sols (containing a marker dye but no drug) show that 77% of the sol had formed a gel at this pH at 1 h, decreasing to about 20% at 5 h as a consequence of gel erosion (Fig. 7). The pharmacokinetic data indicate that these gels are able to function as sustained release vehicles although they appear less structured than those formed at low pH (see for example, Itoh et al., 2006). In contrast, it was not possible to detect gel formation in photographs of the contents of rabbit stomachs after administration of 4 ml of pectin DE31 sol. The

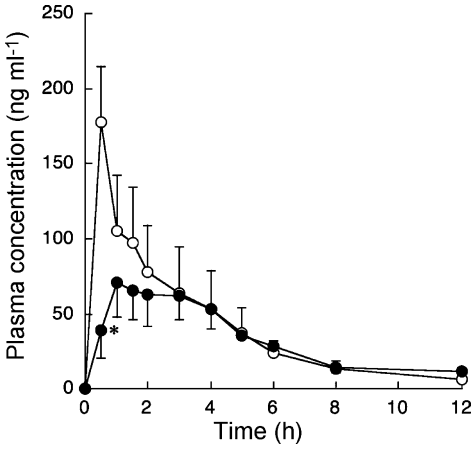

Fig. 6. Plasma concentrations of ambroxol after oral administration of 1.5% (w/v) pectin solutions to gastric-acidity controlled rabbits. (●) Pectin DE9 (pH 5.53) and (○) pectin DE31 (pH 5.70). Each value is the mean ± S.E. of three determinations. *Value is significantly different from pectin DE31 formulation ($p < 0.05$).

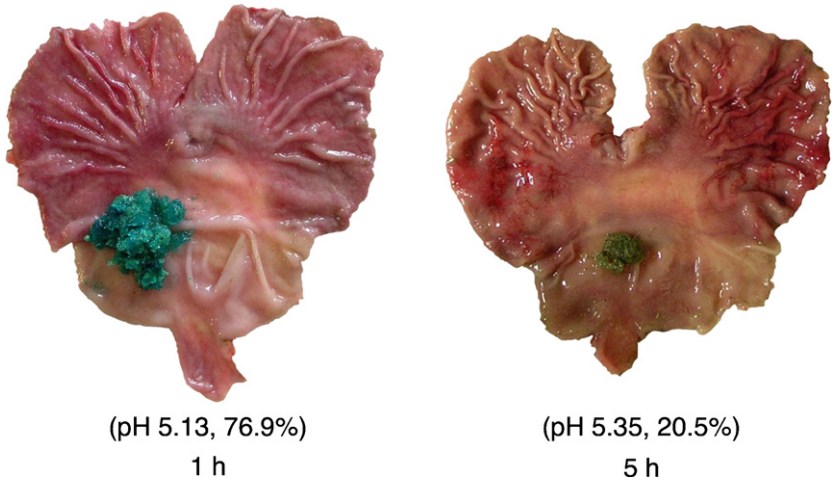

Fig. 7. Photographs showing presence of gels in stomachs of gastric-acidity controlled rabbits 1 and 5 h after oral administration of 1.5% (w/v) pectin sols. Numbers in parenthesis indicate gastric pH and percentage of the gel remaining.

inability of these sols to gel at high pH explains the rapid initial absorption seen in Fig. 6.

The ability of the pectin DE9 formulations to gel in the rabbit stomach at high pH but not in the in vitro diffusion cells even in the presence of added 1.6 mM Ca^{++} ions is possibly a consequence of the presence of other divalent and also trivalent ions in the stomach in addition to calcium ions (Tho et al., 2003). Pectin DE9 is more susceptible than pectin DE31 to cross-linking by these ions (Fishman and Hicks, 2005) hence its more effective in situ gelation.

4. Concluding remarks

This study has shown that 1.5% (w/v) solutions of pectin with a degree of methoxylation of 9% containing a source of calcium in complexed form have potential use as in situ gelling vehicles for oral administration. They exhibit superior in vivo gelling characteristics to formulations prepared with pectin of higher degree of esterification (31%) under conditions in which the hydrogen ion concentration is insufficiently high to effectively release the complexed calcium. It is suggested that the more effective in situ gelling of the low methoxylated pectin in rabbit stomach at high pH may be a consequence of an increased susceptibility to cross-linking by Ca^{++} and other di- or tri-valent ions present in the gastric juice as demonstrated by in vitro experiments. Comparative studies of the pharmacokinetics of ambroxol following oral administration of the pectin formulations have shown a sustained release from formulations containing pectin of the lower degree of esterification.

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