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The effect of taste masking agents on in situ gelling pectin formulations for oral sustained delivery of paracetamol and ambroxol

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Abstract

The aim of this study was to examine the influence of polyhydric alcohols (taste masking agents) on the rheological properties of in situ gelling pectin formulations and on the in vitro and in vivo release of paracetamol and ambroxol from these formulations. Gelation of orally administered pectin solutions containing calcium in complexed form occurred on release of calcium in the acidic environment of the stomach. Inclusion of 10% (w/v) sorbitol in 2% (w/v) pectin sols reduced the viscosity and ensured Newtonian flow properties. Xylitol and mannitol in similar concentrations were less effective in reducing viscosity; sucrose increased viscosity and caused non-Newtonian flow. The in vitro release of paracetamol from 2% (w/v) pectin gels formulated with 10% (w/v) of sorbitol, erythritol, xylitol or mannitol, and of ambroxol from 2% (w/v) pectin gels containing 10% (w/v) sorbitol, followed diffusion-controlled kinetics. Pectin gels (2%, w/v) containing sorbitol (10%, w/v) sustained the release of paracetamol in the rat stomach and bioavailabilities of approximately 90% of those from an orally administered paracetamol syrup were achieved. Sustained release of ambroxol from in situ gelling formulations was achieved with pectin concentrations of 1.5 and 1% (w/v) and a sorbitol concentration of 10% (w/v).

Keywords: In situ gelation; Oral drug delivery; Sustained release; Pectin gels; Taste masking agents; Paracetamol; Ambroxol

1. Introduction

Paracetamol (acetaminophen) is widely used as an analgesic and antipyretic and is usually administered orally in tablet form. For pediatric patients

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and the elderly who have difficulty swallowing solid dosage forms, suppositories (Coulthard et al., 1998; Hansen et al., 1999), syrups (Autret et al., 1994), or chewable tablets (Suzuki et al., 2003) may be more suitable dosage forms. Several oral formulations have been designed to achieve a more sustained delivery of this drug. Endo et al. (2000), for example, achieved a high (90%) bioavailability in rabbits from an orally administered heat-sensitive melting gel containing κ-carrageenan and gelatin as gelling agents. Ambroxol is used in the treatment of bronchitis to improve expectoration and requires three dosings per day for optimum therapeutic efficacy (Vergin et al., 1985). Sustained release formulations based on tablet, pellet or capsule dosage forms have been developed to improve patient compliance. For example, Alighieri et al. (1988) reported sustained release capsules containing lipid matrices, the release of ambroxol from which was controlled by a dialyzing membrane.

We have recently described in situ gelling formulations of the polysaccharides gellan and sodium alginate (Kubo et al., 2003), xyloglucan (Miyazaki et al., 2003), and pectin (Kubo et al., 2004a,b), which were designed to achieve sustained release following oral administration. The xyloglucan formulation was administered as a chilled solution which gelled in the stomach on attainment of body temperature. In situ gelation of the low methoxy pectin solutions was achieved by including a soluble nonionised calcium complex in the solution which breaks down to release free Ca^{2+} ions when the orally administered liquid formulation reaches the acidic environment of the stomach, so ensuring instantaneous gelation. Paracetamol and ambroxol release from both formulations in animal models was sustained over a period of at least 6 h with appreciably lower peak plasma levels.

With liquid formulations or chewable tablets it is often necessary to include taste masking agents, particularly for the administration of paracetamol; sucrose or polyhydric alcohols such as sorbitol are often utilised for this purpose. In the present study we have examined the effects of sucrose and several polyhydric alcohols including sorbitol, erythritol, xylitol and mannitol on the sol–gel transition, rheological properties and release characteristics of the in situ gelling pectin formulations.

2. Materials and methods

2.1. Materials

Pectin (LM-104AS, DE = 31%, Lot 23001-7) was supplied by SANSHO Co., Osaka, Japan. Ambroxol hydrochloride (Lot YT-13) was supplied by YIA Co., Shiga, Japan and paracetamol (acetaminophen) was obtained from Yamanouchi Pharmaceutical Co., Tokyo. Caronal Syrup was from Showa Yakuhin Kako Co. Ltd., Tokyo. D-Sorbitol, xylitol, D-mannitol, sucrose and erythritol were obtained from Wako Pure Chemical Ind. Ltd., Osaka, 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 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) or ambroxol hydrochloride (0.6%, w/v) and D-sorbitol (0, 5, 10, 30 or 40%, w/v) or selected polyhydric alcohol (10%, w/v) were then dissolved in the resulting solution. Solutions of paracetamol (1%, w/v) and ambroxol hydrochloride (0.6%, w/v) were prepared in ultrapure water.

Instantaneous gelation was observed when the pectin sols were added dropwise to simulated gastric fluid (pH 1.2) as a consequence of the release of calcium ions from the soluble calcium complex in the acidic solution.

2.3. 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) using a 1 ml aliquot of the sample. Measurements on each sol were performed in triplicate each taking approximately 30 s.

Gel strengths of pectin formulations with and without added D-sorbitol were measured at 37 °C using a rheometer (CR-500DX, Sun Scientific Co., Tokyo) by the method described previously (Miyazaki et al., 1998; Watanabe et al., 1994). Cylindrical gels of 2% (w/v)

pectin were 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 (26 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. Measurement of in vitro drug release

The release rates of paracetamol and ambroxol were measured by 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 pectin (1, 1.5 and 2%, w/v) loaded with 1.0% (w/v) of paracetamol or 0.6% (w/v) of ambroxol, 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, 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. Comparison was made with Caronal Syrup (containing 1%, w/v of paracetamol) or a 0.6% (w/v) aqueous solution of ambroxol hydrochloride under the same conditions. The concentration of paracetamol or ambroxol in the samples was determined by HPLC as described below.

2.5. Animal experiments

Male Wistar rats, weighing 250–350 g, were fasted for 24 h with free access to water. The sol preparation (0.5 ml) containing 10 mg paracetamol or 3 mg ambroxol hydrochloride was administered orally using a stomach sonde needle for rats (Natume Seisakusho, KN-349D). A stomach sonde needle was also used for oral administration of Caronal Syrup (10 mg paracetamol in 0.5 ml) or the ambroxol hydrochloride solution (3 mg in 0.5 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.

2.6. Paracetamol assay

The plasma samples were separated by centrifugation and assayed by 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. (1981) 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 mm × 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.

2.7. Ambroxol assay

The plasma samples were separated by centrifugation and assayed by HPLC (Shimazu LC-10A with a Shimazu SPD-10A detector at a wavelength of 210 nm) using the method described by Botterblom et al. (1987) with minor modifications. 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 sodium hydroxide and 5 ml of diethyl ether and the sample was vortex-mixed and centrifuged. To supernatant was added 150 μl of 0.01 M hydrochloric acid. 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 C_{18}. A column (20 mm × 3.9 mm i.d.) packed with Waters μBondapak C_{18} was used as a guard column. Elution was carried out with acetonitrile–methanol–0.05 M phos-

phate buffer (0.65:1:3) at a rate of 0.8 ml min^{-1} at 40 °C.

3. Results and discussion

3.1. The influence of polyhydric alcohols on the viscosity of pectin sols

Comparison of Fig. 1a and b shows that in sorbitol-free pectin formulations the presence of calcium in complexed form caused an increase in viscosity, particularly in the case of the 2% (w/v) pectin sol, the flow properties of which also changed from Newtonian to shear thinning behaviour. Although the calcium in these formulations is in the form of a nonionised complex with citrate, there are free Na^+ ions present in the sols (from the sodium citrate) which will interact with the ionised COO^- moieties of the pectin. Whereas Ca^{2+} ions form bridges between carboxylic groups on the same or different pectin chains causing gelation through chain aggregation, Na^+ ions interact with only one carboxylic group. Nevertheless, this interaction has been shown to result in limited inter- and intra-chain bridging (Manunza et al., 1998; Yoo et al., 2003), which is the probable cause of the increased viscosity and change to non-Newtonian flow properties observed in the 2% (w/v) sorbitol-free pectin sols. The presence of 10% (w/v) sorbitol moderates the effect of the calcium complex on sol viscosity and although the viscosity of the 2% (w/v) pectin sols was higher when the sols contained calcium complex, the flow properties of both sols were Newtonian when sorbitol was included in the formulation. The viscosities of each of the three pectin sols (1, 1.5 and 2%, w/v) containing calcium complex decreased following the inclusion of sorbitol in the formulation, whereas in calcium-free solutions of pectin the addition of 10% (w/v) sorbitol caused an increase in viscosity.

Fig. 2a and b show the influence of sorbitol concentration on the flow properties of a 2% (w/v) pectin sol in the presence and absence of complexed calcium. In both systems the viscosity of the pectin sols increased appreciably as the sorbitol concentration was increased from 5 to 40% (w/v). The viscosities of the calcium-free sols containing low concentrations of sorbitol (5 and 10%, w/v) were lower than the equivalent sols containing calcium complex; at higher sorbitol contents viscosities were similar at equivalent sorbitol concentrations. Addition of only 5% (w/v) sorbitol to sols containing calcium complex caused a change of flow properties from shear thinning to Newtonian behaviour and a reduction of viscosity at all shear rates. These changes in viscosity resulting from sorbitol addition have implications for the administration of the sols of this formulation; the lower viscosity improving the ease of swallowing of the solutions.

The polyhydric alcohols xylitol, erythritol, and mannitol increased the viscosity of calcium-free pectin sols to a similar extent as sorbitol when added at the same concentration (Fig. 3). In 2% (w/v) pectin sols containing calcium complex all of these excipients except erythritol changed the flow properties from shear thinning (in polyhydric alcohol-free sols) to Newto-

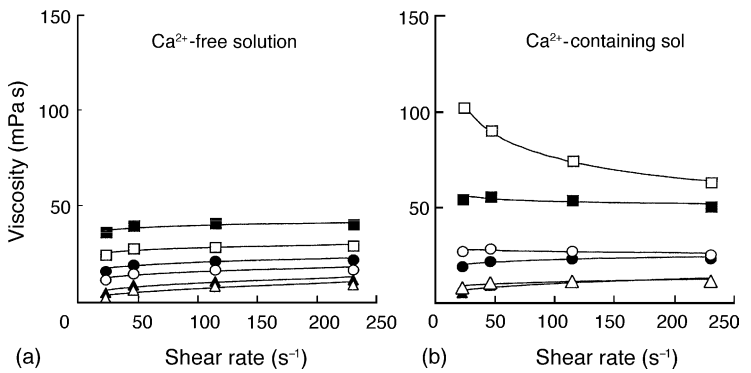

Fig. 1. Effect of sorbitol (10%, w/v) on the viscosity at 20 °C of (a) calcium-free and (b) calcium-containing sols with pectin concentrations: (triangles) 1.0, (circles) 1.5, and (squares) 2.0% (w/v). Closed symbols refer to pectin sols containing sorbitol, open symbols refer to pectin with no added sorbitol. Each value is the mean ± S.E. of three determinations.

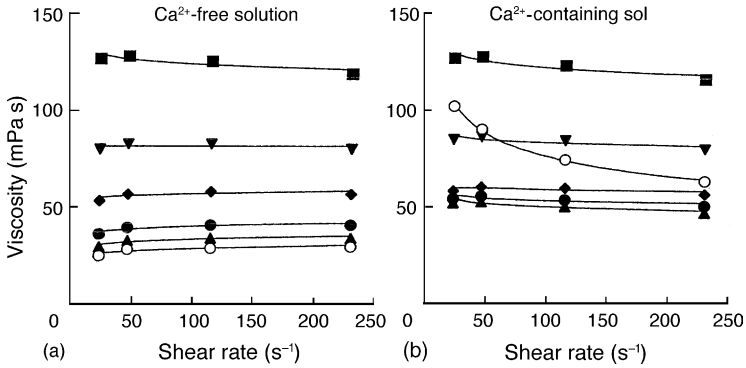

Fig. 2. Viscosity at 20 °C of (a) calcium-free and (b) calcium-containing 2.0% (w/v) pectin sols containing (◯) 0, (▲) 5, (●) 10, (♦) 20, (▼) 30 and (■) 40% (w/v) sorbitol. Each value is the mean ± S.E. of three determinations.

nian flow. The efficiency of the polyhydric alcohols in reducing the viscosity of these sols was in the order sorbitol > xylitol > mannitol > erythritol. A change in xylitol concentration over the range 5–40% (w/v) caused similar changes in the flow properties of a 2% (w/v) pectin sol containing calcium complex to those of sorbitol shown in Fig. 2b. The influence of sucrose on the flow properties of 2% (w/v) pectin sols containing calcium complex is shown in Fig. 4. A considerable increase of viscosity was noted with sucrose concentrations of between 10 and 40% (w/v), all formulations exhibiting pronounced shear thinning characteristics.

Polyhydric alcohols such as sorbitol preferentially bind water molecules causing desolvation around the pectin chains and minimising the hydrogen bonding of water molecules to pectin chains. As a consequence, pectin chains can approach each other more closely and form cross-linked bonds, with a resulting increase of viscosity. In addition, cross linking of pectin chains will also occur by hydrogen bonding between hydroxyl groups of sorbitol and those of the pectin (Chen and Joslyn, 1967). Both of these effects would be expected to lead to an increase of viscosity following the inclu-

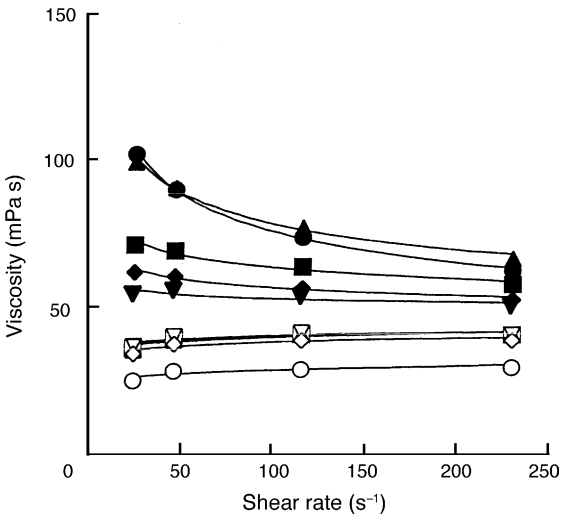

Fig. 3. Effect of taste masking agents (10%, w/v) on the viscosity of calcium-free (open symbols) and calcium-containing (closed symbols) pectin sols (2.0%, w/v) at 20 °C. (circles) no added polyhydric alcohol, (inverted triangles) sorbitol, (triangles) erythritol, (diamonds) xylitol and (squares) mannitol. Each value is the mean ± S.E. of three determinations.

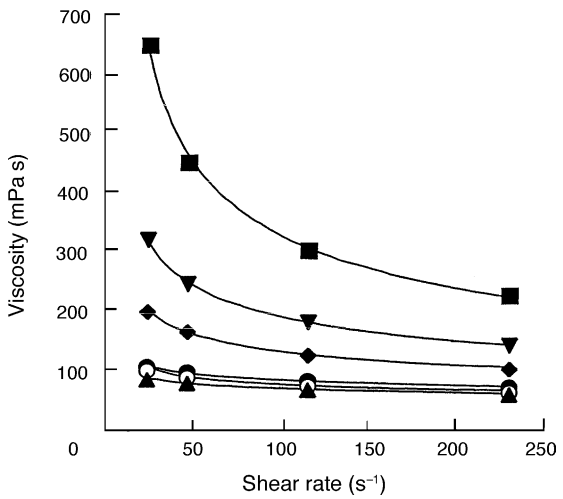

Fig. 4. Viscosity at 20 °C of calcium-containing 2.0% (w/v) pectin sols containing (◯) 0, (▲) 5, (●) 10, (♦) 20, (▼) 30 and (■) 40% (w/v) sucrose. Each value is the mean ± S.E. of three determinations.

sion of sorbitol in the formulation, as was observed in the calcium-free pectin sols (Figs. 1a and 2a). The observed increase of viscosity with increase of concentration of sorbitol (Fig. 2a) is an expected consequence of increased bonding as the number of polyhydric alcohol molecules is increased. The other polyhydric alcohols, mannitol (hexahydric), xylitol (pentahydric), and erythritol (tetrahydric) had similar enhancing effects on the viscosity of the calcium-free sols as the hexahydric alcohol sorbitol (Fig. 3).

The reasons for the observed effects of the polyhydric alcohols on the viscosity of the pectin sols containing complexed calcium are less clear. Fig. 3 shows that addition of 10% of each of these alcohols, except the tetrahydric erythritol, appreciably reduced the viscosity of these sols and caused a change to Newtonian flow. The order of effectiveness of the alcohols in reducing viscosity (sorbitol > xylitol > mannitol > erythritol) is directly related to the number of –OH groups in these molecules suggesting that the effect is related to the hydrogen bonding potential of the alcohol. The exception is of course mannitol, which although hexahydric, has less effect on viscosity than the pentahydric xylitol. However, mannitol has a much lower affinity for water than sorbitol (its solubility is about 3.5 times less than that of sorbitol) although both are hexahydric alcohols. In general, our results have shown a viscosity reducing effect of these alcohols at concentrations of 20% or less, suggesting disruption of the aggregating effect of free Na^+ ions in solution, but a viscosity enhancing effect at higher concentrations (Figs. 2 and 4) presumably as the hydrogen bonding effects of the alcohols discussed above predominate. Fig. 4 shows non-Newtonian flow in sols containing even low concentrations of sucrose, suggesting strong hydrogen bonding and dehydration effects of this di-saccharide as might be expected from its high affinity for water.

3.2. Rheological properties of pectin gels

The influence of sorbitol on the gel strengths of 2% (w/v) pectin gels containing complexed calcium in simulated gastric fluid (pH 1.2) at 37 °C was determined using a simple method that measured the change in load of a probe pushed slowly through the gel. Stress–strain plots (Fig. 5) were typical of those for elastic gels showing a sudden decrease of stress after the maximum, indicative of a brittle system. Values of gel strength (taken as the stress at the point of collapse of the gel structure) measured after equilibration of the gels for 24 h were 84.2, 83.2 and 80.9 kN m^{-2} for sorbitol concentrations of 0, 10 and 20% (w/v), respectively.

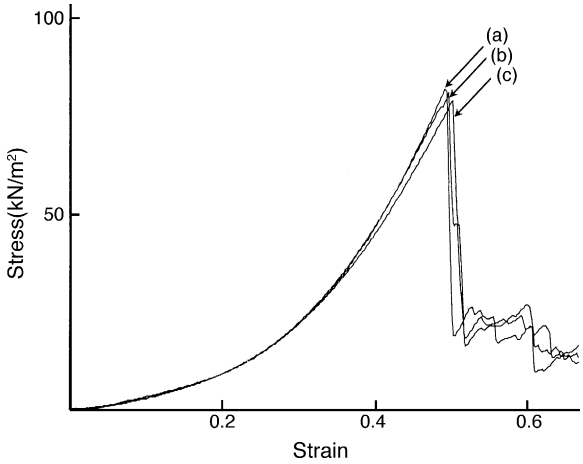

Fig. 5. Rheological properties of 2.0% (w/v) pectin gels containing (a) 0, (b) 10 and (c) 20% (w/v) sorbitol, in simulated gastric fluid at pH 1.2 and 20 °C.

3.3. Release of paracetamol

3.3.1. In vitro release

The release profiles of paracetamol from gels formed by gelation of 2% (w/v) pectin sols with sorbitol concentrations over the range 0–20% (w/v) and containing complexed calcium are compared in Fig. 6 with that from a commercial syrup (Caronal Syrup) containing the same concentration of paracetamol (1%, w/v). 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 gastro-intestinal transit. The pK_a of paracetamol is 9.5 (Florey, 1974) and consequently there will be no change in the state of ionisation of this acidic drug accompanying the pH change. For gels containing 20% (w/v) sorbitol there was a pronounced increase of release after about 3 h; the cumulative amount released from these formulations approached that from Caronal Syrup. No such inflection was observed for gels formed in the presence of 0, 5 and 10% (w/v) sorbitol. Observation of the contents of the donor cell during release measurements showed that the inflection in the plots for release from the formulation containing 20% (w/v) sorbitol

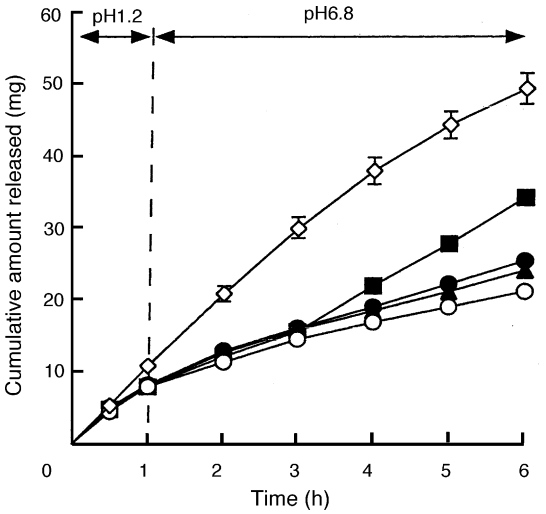

Fig. 6. In vitro release of paracetamol from 2.0% (w/v) pectin sols containing (◯) 0, (▲) 5, (●) 10, and (■) 20% (w/v) sorbitol and (◊) Caronal Syrup, plotted as cumulative release against time. Release was into simulated gastric fluid pH 1.2 for a period of 1 h and subsequently into simulated intestinal fluid pH 6.8. Each value is the mean ± S.E. of four determinations.

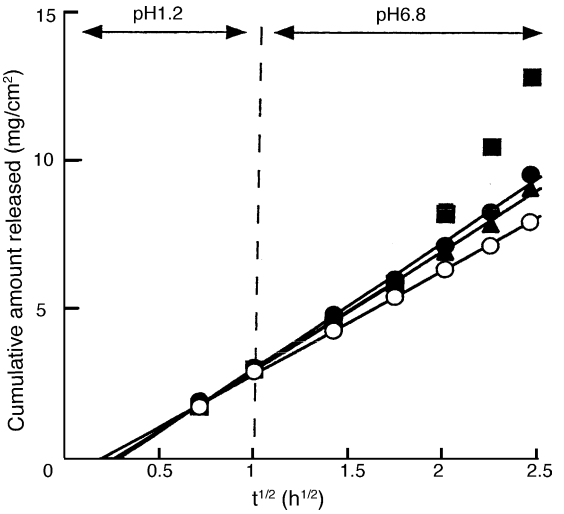

Fig. 7. In vitro release of paracetamol from 2.0% (w/v) pectin sols containing (◯) 0, (▲) 5, (●) 10, and (■) 20% (w/v) sorbitol plotted as cumulative release against square root time. Release was into simulated gastric fluid pH 1.2 for a period of 1 h and subsequently into simulated intestinal fluid pH 6.8. Each value is the mean ± S.E. of four determinations.

coincided with a gel to sol transition, i.e. this gel had insufficient strength to withstand a large decrease of hydrogen ion concentration; gels formed in formulations containing lower sorbitol contents retained their integrity throughout the measurement period.

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 from gels of initial drug concentration C_0 is proportional to the square root of time t:

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

Plots of Q versus $t^{1/2}$ for the release of paracetamol from the pectin gels are shown in Fig. 7. Release from gels with sorbitol concentrations <20%, and over the first 3 h of release from the gel containing 20% (w/v) sorbitol, conformed to Eq. (1) after a short lag period indicating diffusion-controlled release. The diffusion coefficients, D, calculated from the gradients of the plots are given in Table 1. The increasing rate of diffusion as the sorbitol content is increased corresponds to a decrease of gel strength as determined from the rheological measurements.

The in vitro release of paracetamol from 2% (w/v) pectin gels formulated with 10% (w/v) of either erythritol, xylitol or mannitol followed a similar profile to that observed with gels containing 10% (w/v) sorbitol (data not shown). Diffusion coefficients calculated from the linear Higuchi plots are included in Table 1.

3.3.2. *In vivo release*

Plasma drug levels following oral administration to rats of paracetamol (10 mg) from 2% (w/v) sorbitol-

Table 1
Effect of taste masking agents on the in vitro release of paracetamol from 2% (w/v) pectin gels

Taste masking agent	Concentration (%, w/v)	10^6 D (cm^2 s^{-1})
D-sorbitol	0	6.83 ± 0.38
	5	9.00 ± 0.24
	10	10.01 ± 0.25
	20	12.35 ± 0.80 (t < 3 h)
D-mannitol	10	10.33 ± 0.21
Xylitol	10	10.33 ± 0.21
Erythritol	10	10.92 ± 0.16

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

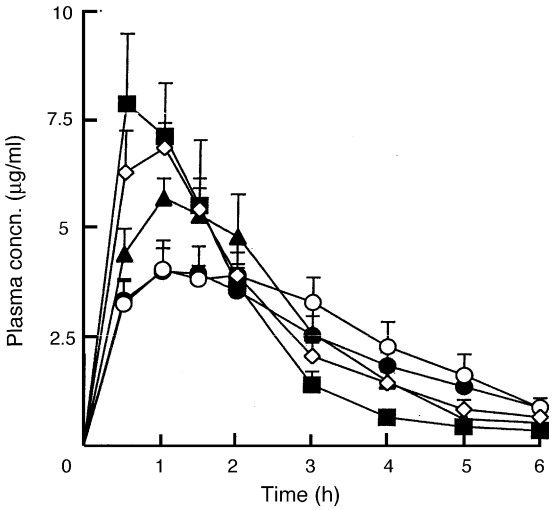

Fig. 8. Effect of taste masking agents on the plasma concentrations of paracetamol in rats after oral administration of 2.0% (w/v) pectin sols containing (◯) 0, (▲) 5, (●) 10, and (■) 20% (w/v) sorbitol, (▲) 10% (w/v) xylitol and (◊) Caronal Syrup. Each value is the mean ± S.E. of five determinations.

free pectin sols, from 2% (w/v) pectin sols containing either sorbitol (10 or 20%, w/v) or xylitol (10%, w/v), and from Caronal Syrup, are compared in Fig. 8. All pectin sols contained calcium in complexed form to ensure in situ gelation in the acidic conditions of the stomach. Observation of the stomach contents 15 min after oral administration of a 1.5% pectin sol showed the presence of a well-defined gel block; it is probable that gelation is instantaneous, as shown from in vitro experiments, but it was not possible to make observations at a shorter time interval than this. 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 (Yamaoka et al., 1981) are summarised in Table 2.

Comparison of release data for gels formulated without sorbitol and those containing 10% (w/v) sorbitol showed no significant difference in behaviour. Both formulations sustained the release of paracetamol as evidenced by lower C_{max}, higher t_{max} and longer mean retention times compared to the commercial syrup. In contrast, paracetamol was absorbed more rapidly from gels in which the sorbitol concentration was 20%, with peak plasma drug concentrations of similar magnitude and at similar times as that of the commercial syrup and with significantly shorter retention times than the formulations with lower sorbitol content. Substitution of 10% (w/v) sorbitol with 10% (w/v) xylitol resulted in release profiles with characteristics intermediate between those of the sorbitol gels and the commercial syrup.

Visual observation of the contents of the rat stomach 3 h after administration of 0.5 ml of the 2% (w/v) pectin sols (containing a marker dye but no drug) showed the presence of gel blocks (see Fig. 9). The amounts of pectin remaining in gel form at this time, expressed as a percentage of the original amount administered, were approximately 68, 64, 57 and 59% for formulations containing 0, 10 and 20% (w/v) sorbitol and 10%

Table 2
Bioavailability parameters of paracetamol and ambroxol from pectin gels formed in situ in rat stomach

Formulation	C_{max} (μg ml^{-1})	t_{max} (h)	AUC (0–6 h) (μg h ml^{-1})	MRT (h)	AUC_{gel}/AUC_{syrup}
Paracetamol					
2% Pectin	4.98 ± 0.55[c]	1.30 ± 0.25	15.68 ± 1.48	2.48 ± 0.18[c]	0.93 ± 0.09
2% Pectin + 10% sorbitol	4.08 ± 0.57[a]	1.40 ± 0.19	14.47 ± 1.93	2.46 ± 0.04[b]	0.86 ± 0.11
2% Pectin + 20% sorbitol	8.57 ± 1.62	0.70 ± 0.12	15.68 ± 2.72	1.67 ± 0.14	0.93 ± 0.16
2% Pectin + 10% xylitol	6.05 ± 0.53	1.30 ± 0.25	16.21 ± 2.33	2.02 ± 0.11	0.96 ± 0.14
Caronal Syrup	7.56 ± 0.58	0.90 ± 0.19	16.84 ± 1.31	1.96 ± 0.08	–
Ambroxol					
1% Pectin	53.30 ± 10.08[a]	1.20 ± 0.46	134.22 ± 18.92[d]	2.72 ± 0.17[c]	–
1% Pectin + 10% sorbitol	58.32 ± 7.62[a]	1.50 ± 0.45	171.18 ± 28.32[c]	2.60 ± 0.09[d]	–
1.5% Pectin	34.70 ± 3.68[a]	1.63 ± 1.13	103.55 ± 13.76[d]	2.65 ± 0.25	–
1.5% Pectin + 10% sorbitol	68.78 ± 12.73[c]	1.13 ± 0.38	180.16 ± 33.38	2.76 ± 0.07[a]	–
0.6% Aq. solution	183.46 ± 33.21	0.50 ± 0.00	298.97 ± 44.20	1.99 ± 0.21	–

[a] $p < 0.005$, [b] $p < 0.001$, [c] $p < 0.05$ and [d] $p < 0.01$, compared with Caronal Syrup or aq. solution as appropriate. Each value represents the mean ± S.E. of 4–5 experiments.

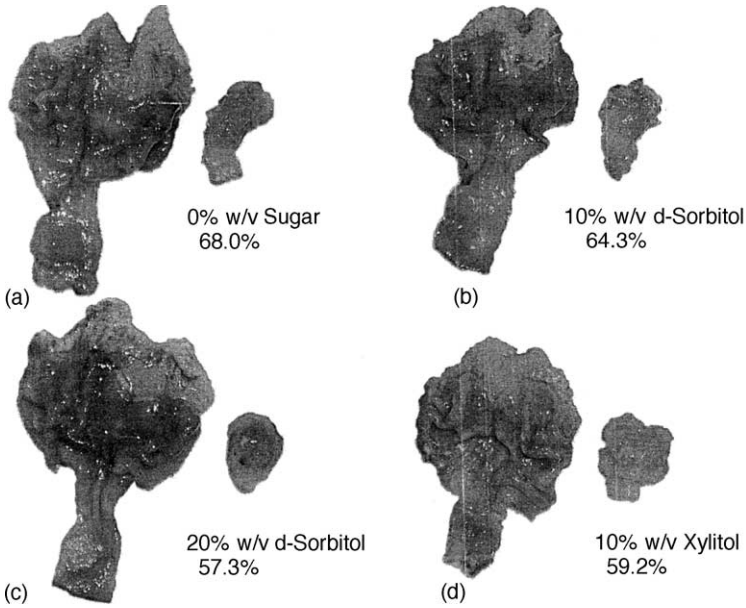

Fig. 9. Photographs showing presence of gels in rat stomach 3 h after oral administration of 2.0% (w/v) pectin sols containing (a) 0, (b) 10, and (c) 20% (w/v) sorbitol and (d) 10% (w/v) xylitol.

(w/v) xylitol, respectively. It is interesting to note that the percentages of remaining gel are in the same rank order as the mean retention times for these formulations suggesting a correlation between release characteristics and gel integrity. The rapid absorption of drug from 20% (w/v) sorbitol gels and the low MRT of these gels compared with those of lower sorbitol content is therefore linked to a more rapidly eroding gel. In vitro release studies showed similar poor gelation characteristics for this formulation.

3.4. Release of ambroxol

3.4.1. In vitro release

The influence of pectin concentration on the release of ambroxol from formulations containing either 0 or 10% (w/v) sorbitol is shown in Fig. 10a. As in release studies on paracetamol, 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. The basic group of ambroxol ($pK_b = 7.16$, Heinanen and Barbas, 2001) will be fully ionised at pH 1.2 and approximately 50% ionised at pH 6.8; this change of ionisation did not, however, cause any significant discontinuities in the release curves at 1 h. Pronounced inflections were, however, noted in release curves for 1% (w/v) pectin gels containing 10% (w/v) sorbitol after approximately 3 h. Observation of the contents of the donor cells during release from this formulation showed that the cause of this inflection was the partial reversion to the sol state, which occurred at a similar time. Similar, but less pronounced changes of slope were noted in the plots for release from 1.5 and 2% (w/v) gels.

Analysis of the data, according to Eq. (1) produced the plots shown in Fig. 10b, where departure from linearity is clearly seen after release periods of between 3 and 4 h. Diffusion coefficients calculated from linear regions of the Higuchi plots are summarised in Table 3. The decrease of D with increasing pectin concentration in sorbitol-free formulations is an expected con-

Table 3
Effect of sorbitol on the in vitro release of ambroxol from pectin gels

Formulation	$10^6\ D$ ($cm^2\ s^{-1}$)
1% Pectin	5.74 ± 0.19 ($t < 4$ h)
1.5% Pectin	4.56 ± 0.18
1.5% Pectin + 10% sorbitol	9.03 ± 0.90 ($t < 3$ h)
2% Pectin	4.37 ± 0.21
2% Pectin + 10% sorbitol	6.92 ± 0.46 ($t < 3$ h)

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

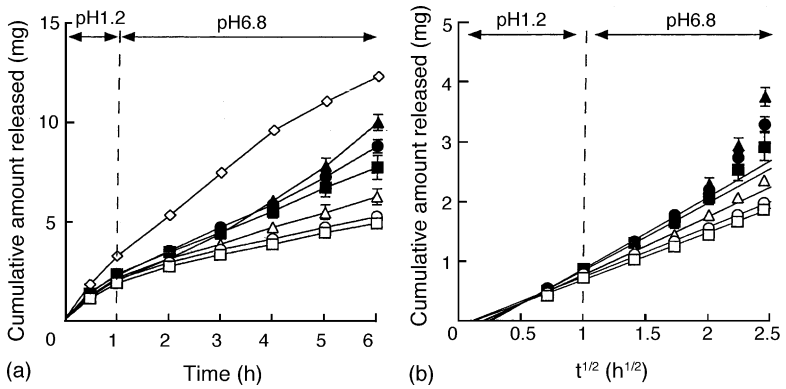

Fig. 10. Effect of sorbitol (10%, w/v) on the in vitro release of ambroxol from pectin sols of concentration: (triangles) 1.0, (circles) 1.5, and (squares) 2.0% (w/v) plotted as cumulative release against (a) time and (b) square root of time. Closed symbols refer to formulations containing sorbitol, open symbols refer to sorbitol-free formulations; (◊) aqueous solution containing ambroxol hydrochloride (0.6%, w/v). 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.

sequence of an increase of gel strength. Inclusion of 10% (w/v) sorbitol in the formulation resulted in alteration of gel structure such that the release of drug was enhanced, as evidenced by the increased diffusion coefficients for the 1.5 and 2% (w/v) pectin gels in the presence of sorbitol (*D* was not calculated for the 1% (w/v) pectin gel containing sorbitol because of the curvature of the plot).

Comparison of diffusion coefficients of ambroxol and paracetamol from 2% (w/v) pectin gels showed higher values for the latter from formulations with both 0 and 10% sorbitol contents.

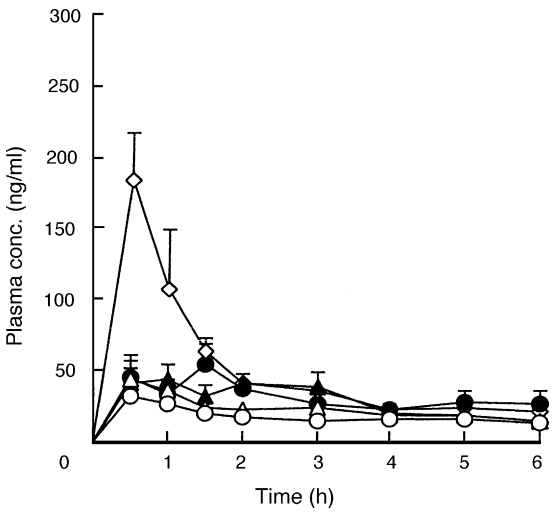

Fig. 11. Effect of sorbitol (10%, w/v) on the plasma concentrations in rats after oral administration of ambroxol from pectin sols of concentration: (triangles) 1.0, and (circles) 1.5% (w/v). Closed symbols refer to formulations containing sorbitol, open symbols refer to sorbitol-free formulations; (◊) aqueous solution containing ambroxol hydrochloride (0.6%, w/v). Each value is the mean ± S.E. of 4–5 determinations.

3.4.2. In vivo release

Plasma drug levels following oral administration to rats of ambroxol (3 mg) from 1.5 and 1% (w/v) sorbitol-free pectin sols, from 1.5 and 1% (w/v) pectin sols containing 10% sorbitol, and from an aqueous solution of ambroxol (3 mg in 0.5 ml) are compared in Fig. 11. Bioavailability parameters for these formulations are compared in Table 2 and show a sustained release of drug from all gel formulations when compared with the solution, with no significant effect of sorbitol on the in vivo release characteristics. Table 2 shows a more sustained release from 1.5% compared to 1% (w/v) sorbitol-free pectin gels (lower C_{max} and higher t_{max}).

4. Concluding remarks

Pectin sols formulated with a source of calcium in complexed form formed gels in the stomach of rats following oral administration, when the calcium ions were released in the acidic environment. This study has

demonstrated that 2% (w/v) pectin gels sustained the release of paracetamol in the rat stomach and bioavailabilities of approximately 90% of those from an orally administered paracetamol syrup were achieved. Sustained release of ambroxol from in situ gelling formulations was obtained with pectin concentrations of 1.5 and 1% (w/v) and a sorbitol concentration of 10% (w/v).

Inclusion of sorbitol in these formulations to improve the taste is an effective way of reducing their viscosity and ensuring Newtonian flow properties, both of which aid the swallowing of the sols. Satisfactory flow of 2% (w/v) pectin sols was achieved with sorbitol concentrations of 5 or 10% (w/v), higher amounts resulted in undesirable increases of viscosity. A change from shear thinning to Newtonian flow was also achieved when sorbitol was replaced with either xylitol or mannitol (10%, w/v), although these polyhydric alcohols were less effective in reducing the viscosity of 2% (w/v) pectin sols. In contrast, erythritol was ineffective in changing flow properties, and the inclusion of sucrose (10–40%, w/v) in the formulation was associated with large increases of viscosity and pronounced non-Newtonian flow properties. No significant change of in vivo release characteristics for either paracetamol or ambroxol was noted when 10% (w/v) sorbitol was included in the pectin sols, but 20% (w/v) sorbitol caused more rapid release of paracetamol as a consequence of a weaker gel structure.

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