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Oral Sustained Delivery of Theophylline and Cimetidine from In Situ Gelling Pectin Formulations in Rabbits

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ABSTRACT The aim of this study was to evaluate the potential of an in situ gelling pectin formulation as a vehicle for the oral sustained delivery of theophylline and cimetidine. In vitro studies demonstrated diffusion-controlled release of theophylline from 1, 1.5, and 2% w/v pectin gels. Release of this drug from 1.5% w/v pectin gels formed in situ in rabbit stomach was sustained over a period of 12 hours giving a theophylline bioavailability some seven fold higher than when administered from a commercial syrup. In contrast, interactions between cimetidine and pectin led to weak gelation of the pectin sols that prevented any meaningful determination of in vitro release characteristics. Similarly, in vivo release profiles from pectin formulations containing cimetidine were similar to that from a solution of this drug in buffer, indicative of weak gelation. Examination of the content of the rabbit stomach 5 hours after administration of 1.5% w/v pectin sols containing drug confirmed gel formation, but gels containing cimetidine were noticeably softer than those containing theophylline.

KEYWORDS Pectin gels, In situ gelation, Oral drug delivery, Sustained release, Theophylline, Cimetidine

INTRODUCTION

We have previously reported the potential of in situ gelling pectin gels for the sustained delivery of paracetamol (Kubo et al., 2004a) and ambroxol (Kubo et al., 2004b). Low methoxy pectins (degree of esterification <50%) such as those used in these formulations readily form gels in aqueous solution in the presence of free Ca^{2+} ions, which cross-link the galacturonic acid chains in a manner described by the "egg-box" model (Dumitriu et al., 1996). Gelation of the orally administered liquid formulations was ensured by the inclusion of calcium ions in the formulation as a soluble complex designed to break down to release free calcium ions on encountering the acidic environment of the stomach. In our earlier study (Kubo et al., 2004b), we showed that the inclusion of sorbitol (as a taste-masking agent) in the pectin formulations had appreciable

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effects on the flow and release characteristics of pectin formulations. It was possible to formulate effective vehicles without this excipient, which sustained the release of paracetamol in rat over a period of at least 6 hours. This article explores the potential of these vehicles for the oral administration of theophylline and cimetidine. Theophylline was selected as a representative drug for development as a sustained release oral dosage form because of its short elimination half-life, especially in children. Cimetidine is currently supplied as a suspension in combination with sodium alginate (for example, Algitec and Tagamet Dual Action, GlaxoSmithKline, Middlesex, UK) for the suppression of postprandial reflux, and there is potential also for a sustained release formulation of this drug.

The influence of these drugs on the gelling properties of the pectin sols and on the in vitro and in vivo release characteristics of these drugs has been compared.

MATERIALS

Pectin (LM-104AS, DE=31%, Lot 23001-7) was supplied by SANSHO Co. (Osaka, Japan). Theophylline and cimetidine were obtained from Wako Pure Chemical Ind. Ltd. (Osaka), and a commercially available product, Theo-Dur™ Dry Syrup (10 mg mL^{-1}), from Mitsubishi Kasei Co. (Tokyo). 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 to 50°C while stirring. Appropriate amounts of theophylline or cimetidine were then dissolved in the resulting solution. A 1% w/v solution of cimetidine in phosphate buffer at pH 5.0 was also prepared.

Measurement of In Vitro Drug Release

Release rates were measured 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 (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 fluid (pH 1.2) or intestinal (pH 6.8) fluid (as specified for the JP XIV disintegration test) was initially placed in the receptor compartment. However, it should be noted that although a pH of 1.2 was used as a representation of the gastric acidity, there is evidence that the pH of the rabbit stomach may not be as low as this, and consequently inferences from in vitro to in vivo data should be tempered with caution. After one hour, the receptor solution was changed to a simulated intestinal fluid at pH 6.8 to mimic gastrointestinal transit (Chaw et al., 2001).

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. The drug concentration of the samples was determined using a spectrophotometer at wavelengths of 274 nm (theophylline) and 220 nm (cimetidine).

Animal Experiments

White male rabbits weighing 3.0 to 3.3 kg were fasted for 24 hours before the experiments but allowed free access to water. In addition to the fasting process, which ensured that very little food was present in the stomach (from visual observation), a yoke was used to avoid the possibility of coprophagy. The sol preparation (4 mL) containing 40 mg theophylline was orally administered using a stomach sonde needle for rabbits (Natume Seisakusho, KN-342). A stomach sonde needle was also used for oral administration of the commercial Theo-Dur™ Dry Syrup (40 mg in 4 mL) and a solution of cimetidine (80 mg in 8 mL) in buffer at pH 5.0. Gels containing cimetidine were produced in situ by oral administration of 8 mL of the pectin solution containing 80 mg of drug. At given intervals, a blood sample was taken from the ear vein and analyzed as described below.

The protocols for the animal experiments were previously approved by the Animal Ethics and Research Committee of the Health Sciences University

of Hokkaido. The statistical significance of the results was assessed by the Student's t test and results are presented as the mean±standard error of the mean.

Determination of Drugs

Theophylline

The plasma samples were separated by centrifugation and assayed for theophylline by HPLC (Shimazu LC-10A with a Shimazu SPD-10A detector at a wavelength of 273 nm) using the method described by Schreiber-Deturmeny and Bruguerolle (1996) with minor modifications. To 0.05 mL of plasma was added 50 μL of caffeine solution (15 μg/mL^{-1}) as internal standard and 20 μL of 20% perchloric acid, and the sample was vortex-mixed and centrifuged. The supernatant was passed through a Millipore filter (0.45 μm) and directly injected onto a 250 × 4.6 mm i.d. column, packed with Inertsil-ODS. Elution was carried out with acetonitrile-tetrahydrofuran-concentrated acetic acid-distilled water (100:20:5:875) at a rate of 1.0 mL min^{-1} at 40°C.

Cimetidine

The HPLC assay of cimetidine was based on the methods described by Russel et al. (1994) and Kelly et al. (1995) with minor modifications. To 100 μL of plasma was added 100 μL of ranitidine solution (10 μg mL^{-1}) as internal standard, 100 μL of 1 M sodium hydroxide, 100 μL of saturated solution of potassium carbonate, and 1 mL of ethyl acetate-isoamyl alcohol (96:4) and the sample was vortex-mixed and centrifuged. To 100 μL supernatant was added 100 μL of 0.01 M hydrochloric acid. After shaking and centrifugation, the aqueous phase was passed through a Millipore filter (0.45 μm) and injected onto a 250 × 4.6 mm i.d. column, packed with Inertsil-ODS2. The HPLC instrument was the same as that described previously, with detection at 228 nm. Elution was carried out with 0.01 M phosphate buffer at pH 6.2 containing 2.5 g L^{-1} heptanesulfonic acid:acetonitrile (75:25) at a rate of 1.0 mL min^{-1} at 40°C.

RESULTS AND DISCUSSION

In Vitro Drug Release

The release profiles of theophylline from 1.0, 1.5, and 2% w/v gels are compared in Fig. 1 with that from

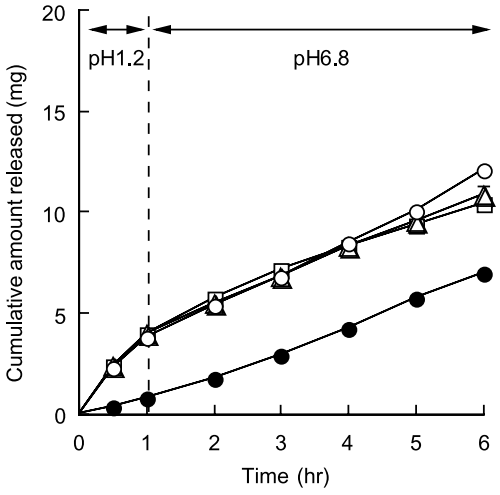

FIGURE 1 **Cumulative Release of Theophylline as a Function of Time from Pectin Sols of Concentrations (% w/v) (○) 1.0, (△) 1.5, (□) 2.0, and (●) Theo-Dur Dry Syrup. All Formulations Initially Contained 40 mg Theophylline. Release was into Simulated Gastric Fluid pH 1.2 for a Period of 1 hour and Subsequently into Simulated Intestinal Fluid pH 6.8. Each Value is the mean ± S.E. of 3 Determinations.**

Theo-Dur™ Dry Syrup, which is a reconstituted suspension of microparticles (200 μm) of sustained release matrix granulated with D-mannitol in water. The receptor solutions were changed after 1 hour from simulated gastric fluid at pH 1.2 to a simulated intestinal fluid at pH 6.8 to mimic gastrointestinal transit. Gels form in the donor compartment due to rapid diffusion of H^+ ions from the receptor buffer solution causing release of complexed Ca^{2+} ions in the formulation. Observation of the contents of the donor cells during release measurements showed the presence of gels throughout the release experiment.

The release data over the whole time period were analyzed according to the treatment proposed by Higuchi (1962) for drug release from semisolid vehicles containing dissolved drug. For the initial 50% to 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:

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

Plots of Q vs. $t^{1/2}$ for the release of theophylline from the pectin gels are shown in Fig. 2. Release from gels at each of the pectin concentrations conformed to Eq. 1 after a short lag period indicating diffusion-controlled release. The diffusion coefficients, D, (10^{-6} cm^2 s^{-1})

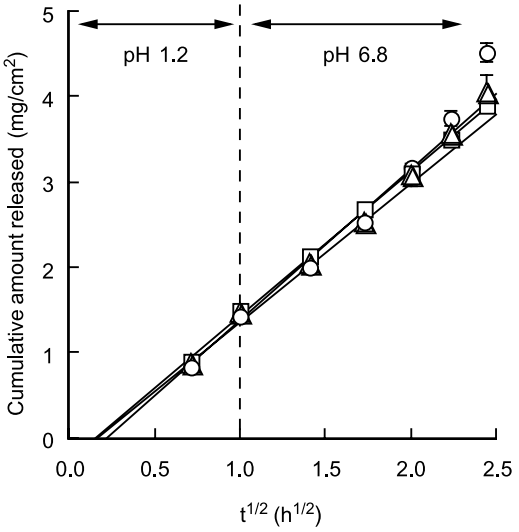

FIGURE 2 **Cumulative Release Per Unit Area, *Q*, of Theophylline as a Function of Square Root of Time from Pectin Sols of Concentrations (% w/v) (○) 1.0, (△) 1.5, and (□) 2.0. Release was into Simulated Gastric Fluid pH 1.2 for a Period of 1 hour and Subsequently into Simulated Intestinal Fluid pH 6.8. Each Value is the mean ± S.E. of 3 Determinations.**

calculated from the gradients of the plots for 1.0, 1.5, and 2.0% w/v gels were 5.66±0.47, 6.87±0.92, and 6.25±0.13 (n=3±S.E.) respectively. There is evidence of departure from linearity at longer release times in the plot of release from 1% w/v gels and the diffusion coefficient calculated for this gel should be treated with some caution.

Figure 3 compares the release profiles of cimetidine from 1.0, 1.5, and 2% w/v gels with that from a 1% w/v solution of cimetidine in phosphate buffer at pH 5.0. The release from the pectin gels shows apparent zero order release characteristics. However, observation of the contents of the donor compartment showed only partial gel formation during the first hour of the study and complete reversion to sol form when the pH of the receptor compartment was changed to pH 6.8. Consequently, the release data for this drug were not analyzed further.

The reason for the lack of gelation of pectin sols loaded with cimetidine is thought to be a consequence of interaction between this drug and the pectin molecules. Gelation of pectin in the formulations of this study normally arises through the cross-linking of the galacturonic acid chains by the free calcium ions released in acid solution, in a manner described by the "egg-box" model (Dumitriu et al., 1996). However, the cimetidine (pK_a=6.8) present in the sol in the donor compartment during the first hour of measurement will be fully ionized (pH of sol=3), and interaction between the cationic $-N^+$ of cimetidine and the anionic COO^- of the pectin, which is approximately 76% ionized (pK_a=3.5), will occur. Such interaction is clearly sufficient to reduce the cross-linking by the divalent calcium ions to an extent that significantly disrupts the gelation process. Although the pH of the donor solution was increased to 6.3 on replacing the buffer in the receptor compartment after 1 hour the cimetidine was still predominantly in its ionized state at this pH so preventing gelation. Strong chemical interaction between cimetidine and the carboxylic acid groups of Carbopol 934 have been reported (Tournier et al., 1988). It is interesting to note that gelation of sols of other polysaccharides such as sodium alginate and gellan gum is not similarly disrupted in the presence of cimetidine (Miyazaki et al., 2001) even though the gelation process also involves cross-linking by calcium ions. Rheological studies of drug-free polysaccharide gels (Kubo et al., 2003, 2004b) have indicated gel strengths (kN m^{-2}) of 190.8, 79.9, and 33.9 for gellan (1% w/v), sodium alginate (1.5 % w/v) and pectin (1.5 % w/v) respectively. The very much weaker pectin gels are clearly more susceptible to interaction with cimetidine than either alginate or gellan gels of similar concentrations.

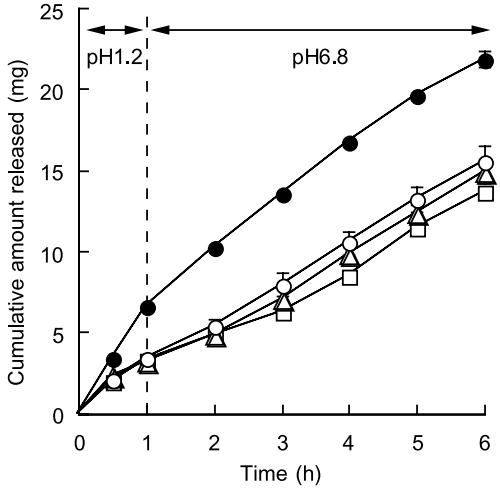

FIGURE 3 **Cumulative Release of Cimetidine as a Function of Time from Pectin Sols of Concentrations (% w/v) (○) 1.0, (△) 1.5, (□) 2.0, and (•) Cimetidine Solution (pH 5.0). All formulations Initially Contained 40 mg Cimetidine. Release was into Simulated Gastric Fluid pH 1.2 for a Period of 1 hour and Subsequently into Simulated Intestinal Fluid pH 6.8. Each Value is the mean ± S.E. of 3 to 4 Determinations. [Data for Cimetidine Solution from Tournier et al. (1988)].**

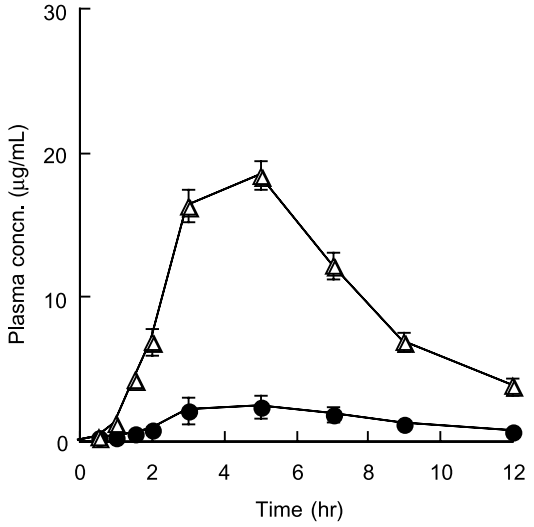

FIGURE 4 **Plasma Concentrations of Theophylline in Rabbits after Oral Administration of (△) 1.5%w/v Pectin Sols and (●) Theo-Dur Dry Syrup. All Formulations Initially Contained 40 mg Theophylline. Each Value is the mean ± S.E. of 4 Determinations.**

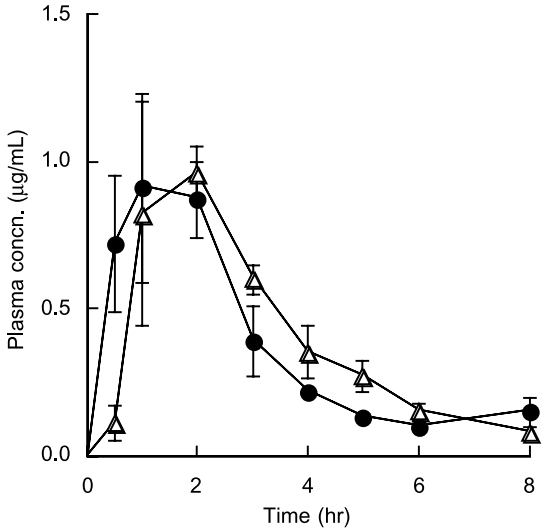

FIGURE 5 **Plasma Concentrations of Cimetidine in Rabbits after Oral Administration of (△) 1.5%w/v Pectin Sols and (●) Phosphate Buffer Solution of pH 5.0. All Formulations Initially Contained 80 mg Cimetidine. Each Value is the mean ± S.E. of 3 to 4 Determinations. [Data of Phosphate Buffer Solution of pH 5.0 from Tournier et al. (1988)].**

No such interaction will occur when either the weakly ionized drug theophylline or the weakly acidic drug paracetamol are included in the pectin sols; these sols form gels in the presence of free calcium ions, which retain their structural integrity throughout the release experiments. However, it is interesting to note that the incorporation of the ambroxol into pectin gels does not result in disruption of gel structure (Kubo et al., 2004b). Ambroxol is a basic drug with similar ionization characteristics to cimetidine [$pK_b = 7.16$ (Heinanen & Barbas, 2001)] and might be expected to interact with the carboxylic acid residues of pectin in a similar manner. However, the ionizable $-NH_2$ moiety of this drug is in close proximity to the ring structures rather than located at the end of a chain as in cimetidine, and this may hinder its interaction with the pectin chains. In addition, the concentration of ambroxol in the in vitro release studies reported previously was only 0.3% w/v compared with a concentration of cimetidine of 1% w/v in the present study.

In Vivo Release

Plasma drug levels following oral administration to rabbits of theophylline (40 mg in 4 mL) from 1.5 w/v pectin sols containing sodium citrate and calcium chloride, and from the Theo-Dur™ Dry Syrup are compared in Fig. 4. In situ 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). The area

TABLE 1 **Bioavailability Parameters of Theophylline and Cimetidine Administered from 1.5% w/v Pectin Gels Formed In Situ in Rabbit Stomach**

Dosage form	C_{max} (μg mL^{-1})	t_{max} (h)	AUC[a] (μg h mL^{-1})	MRT (h)	*n*
Theophylline					
1.5% w/v pectin	19.20 ± 0.71[b]	4.50 ± 0.50	116.09 ± 3.31	5.70 ± 0.15	4
Theo-Dur™ Dry Syrup	2.35 ± 0.82	5.00 ± 0.00	16.34 ± 5.69	6.03 ± 0.18	4
Cimetidine					
1.5% w/v pectin	1.07 ± 0.20	1.67 ± 0.33	3.15 ± 0.39	2.92 ± 0.27	3
Cimetidine soln	1.17 ± 0.18	1.38 ± 0.55	2.96 ± 0.34	2.58 ± 0.36	4

[a] AUC theophylline 0–12 h; cimetidine 0–8 h.
[b] $p < 0.001$ compared Theo-Dur™ Dry Syrup.

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 summarized in Table 1. The theophylline bioavailability was very much higher when released from the pectin gel than from the Theo-Dur™ Dry Syrup, although the mean residence times and t_{max} values were similar with the two dosage forms.

Comparison of the in vivo release profiles for cimetidine (80 mg in 8 mL) from 1.5% w/v pectin sols containing sodium citrate and calcium chloride and from a solution of cimetidine in buffer at pH 5.0 shows similar C_{max} and t_{max} values (see Fig. 5) and bioavailabilities (Table 1). The similarity of release profiles suggests that the cimetidine is released from weak gels or even that gelation of the pectin sols is inhibited by the presence of cimetidine.

Examination of the contents of the rabbit stomach following administration of 4 and 8 mL of 1.5% w/v pectin sol containing a marker dye (but without drug) showed that approximately 71% and 73% of the gel remained at 5 hours after administration, respectively (Fig. 6). Examination of stomach contents after administration of 1.5% w/v pectin sols containing theophylline and cimetidine showed that approximately 70% and 60% gel was present at 5 hours, respectively. However, although these observations show in situ gel formation in the presence of cimetidine the gels formed were appreciably softer than those containing theophylline, which may explain the similarity of the release profile of cimetidine from these gels and that from buffer solution.

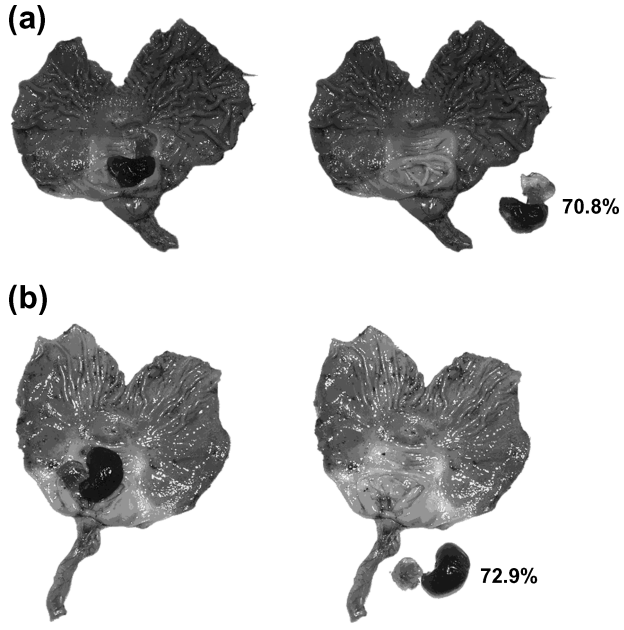

FIGURE 6 **Photographs Showing Presence of Gels in Rabbit Stomach 5 hours After Oral Administration of (a) 4 mL and (b) 8 mL of 1.5% w/v Pectin Sols.**

CONCLUSION

Pectin sols formulated with a source of calcium in complexed form will form gels when the calcium ions are released in the acidic environment of the stomach of rabbits after oral administration. We have shown in this study that in situ gelling formulations with a pectin content of 1.5% w/v sustained the release of theophylline in the rabbit stomach over a period of 12 hours. The bioavailability of theophylline when released from this vehicle was about seven fold higher than when administered from a commercial syrup. Examination of the stomach contents after administration of pectin sols containing theophylline confirmed in situ gelation and showed that 70% gel remained after 5 hours.

However, these formulations are less suitable as vehicles for the release of cimetidine. Whereas in vitro studies of theophylline release showed a diffusion controlled release mechanism, it was not possible to conduct meaningful in vitro release studies on cimetidine because of interactions between this drug and pectin, which hindered gelation and led to the formation of very weak gels that were unable to maintain their integrity on change of pH. In vivo release profiles of cimetidine from orally administered pectin sols were similar to that from a solution of this drug in buffer, suggesting weak in situ gelation. Visual observation of the stomach content after administration of pectin sols containing cimetidine confirmed the presence of weak gels.

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